

soil structure/soil biota interrelationships



**International Workshop on Methods of Research
on Soil Structure/Soil Biota Interrelationships,
held at the International Agricultural Centre,
Wageningen, The Netherlands, 24–28 November 1991**

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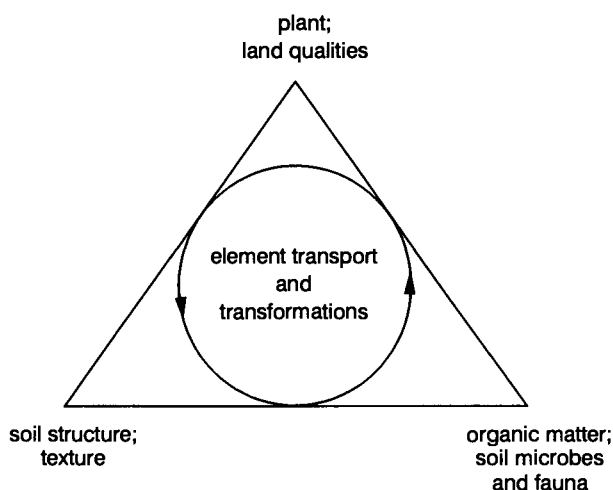
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Editors' Introduction

The aim of the conference on Methods of Research on Soil Structure/Soil Biota Interrelationships, which we organized 24–28 November, 1991, was to provide a thorough review of established and innovative methods of study and assessment at the microscopic scale, that can be used in all aspects of research on this subject in soil ecology and soil science. It appears that much research on the transformation and transport of elements such as carbon and nitrogen is either concerned with soil biota/plant relationships or with soil structure/plant relationships, but very few studies explicitly take the interrelationships between soil structure and soil biota into account:

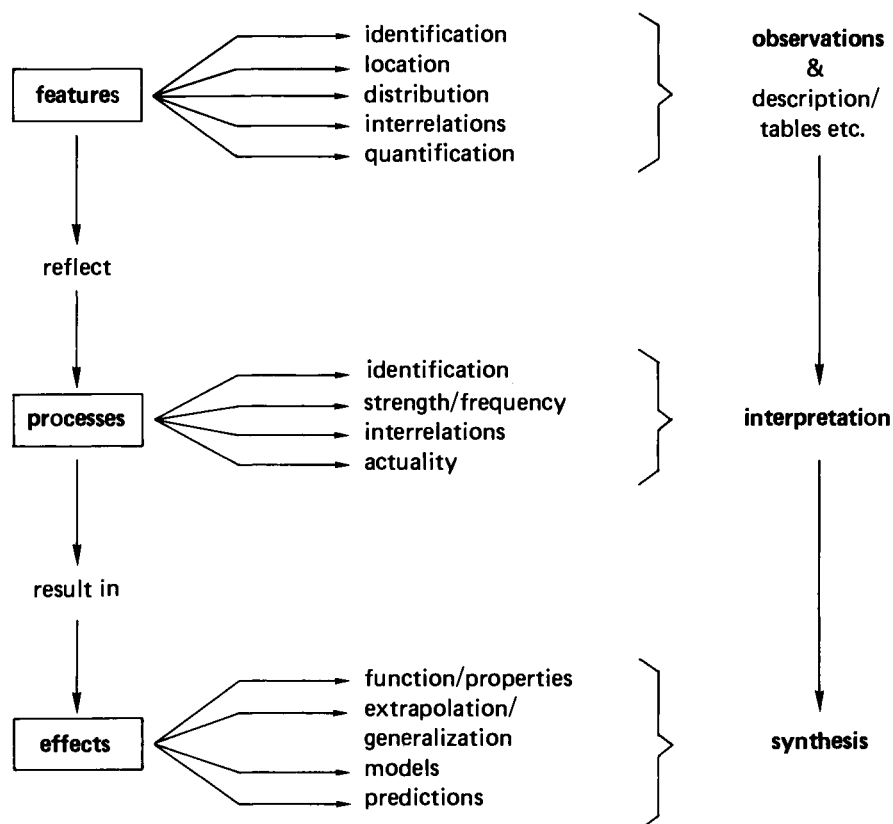


Since both in reduced input agriculture and in (semi-)natural ecosystems the plant depends on the contribution of the soil biota to the formation of soil structure and to the release of nutrients, it is obvious that all aspects of the triangle deserve adequate consideration. This calls for interdisciplinary research among soil microbiologists, soil zoologists, soil micromorphologists, soil physicists and root ecologists.

It appears, however, that state-of-the-art research in these disciplines is often carried out on different scales, depending, e.g., on the size of the biota that is studied or, rather trivial, on the technical equipment available. There is also insufficient knowledge on techniques used in sister disciplines and on the prospects of using those in one's own field of expertise. So one of the chal-

lenges we face is to develop an adequate methodology for the study of soil structure/soil biota interrelationships and for the translation of results from such research to plant performance and land qualities, as indicated in the figure.

Dealing with this challenge different levels of adequate methodology can be distinguished:



The first level concerns the collection of the basic data: observation, description and quantification of primary features. Focussing on methodology the question is: how do we observe, describe and quantify these features, the interaction between soil structure and soil biota, in situ. The second level deals with the arrangement of diagnostic features into processes. The question here is: how do we translate observed soil features into biologically mediated processes. The third level concerns the translation of processes into effects. The basic question is: how to evaluate effects of the features and processes on soils and plants. This set-up was chosen for the workshop and the articles in the proceedings are arranged accordingly.

Although the conference was on innovative methodology in the first place, we also received a number of excellent contributions in which conventional methods were applied. It was therefore decided not to restrict the proceedings entirely to methodology.

The response we have had from the scientific community, both in terms of participation and in terms of manuscripts received, has convinced us that the conference was timely and relevant. The financial support of the sponsors, mentioned elsewhere in this volume, created the possibility to participate for most of the invited speakers and for a considerable number of scientists from developing countries.

Finally, we are confident that the proceedings, like the conference itself, will stimulate co-operation among scientists interested in soil structure and soil biota, to the benefit of application of knowledge in environmentally sound plant production and use of land qualities.

Wageningen, 19 November 1992

L. BRUSSAARD
M.J. KOOISTRA

Sponsors



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Co-operative Research Project on
Biological Resource Management of the
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Netherlands Integrated Soil Research Programme



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International Agricultural Centre

Preface

Reductions of chemical and energy inputs to agriculture could lead to more economical production systems while minimising pollution. This might be achieved by modifying farming systems (e.g. reduction of tillage), the use of improved efficiency organic fertilisers, and the partial replacement of crop protection chemicals with micro-organisms (including those which have been genetically modified).

Theme 1 of the OECD Co-operative Research Project on Biological Resource Management 1990–1994, Modification of plant/soil/microbial interactions to reduce inputs in farming systems, focuses on the role of micro-organisms in these managed ecosystems and their relevance to crop production and protection.

Particular priorities are to:

- Improve existing and develop new detection and assessment methodologies for microbes.
- Identify physicochemical aspects of the soil environment which regulate microbial function.
- Determine population dynamics and survival of soil and rhizosphere communities.
- Analyse the potential of sustainable agriculture systems using inter-disciplinary approaches.

In a planning meeting which was held in Wageningen in the summer of 1990, the following topics were identified as priorities for the Theme to fund fellowships and workshops:

- (1) The soil environment and microbes.
- (2) Plant effects on micro-organisms.
- (3) The soil and plant environment in relation to fertilizer use.
- (4) Food webs and soil fauna.
- (5) Soil quality and biodiversity.
- (6) Biocontrol of pests and diseases.

When it was indicated that Professor Lijbert Brussaard and colleagues were to arrange a workshop on the Topic in Wageningen in 1991, it was obvious that this should be supported by OECD. From the programme arranged, I am sure that our decision to finance the attendance of a large proportion of the overseas speakers was fully vindicated.

In future workshops, we will be endeavouring to cover the remaining topics

and will meet at varying locations with the network of participating countries (Australia, Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, New Zealand, Norway, Portugal, Spain, Sweden, Switzerland, Turkey, United Kingdom, United States at the time of this publication).

It is critical that sustainability of agricultural systems be addressed on interdisciplinary and international bases. Hopefully then reduction in inputs will be achieved in a manner which maintains profitability for farmers whilst protecting the environment, particularly the soil itself on which agricultural productivity depends.

J.M. LYNCH

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The International Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships was sponsored, in part, by the Organisation of Economic Co-operation and Development (OECD). Financial support for this workshop came from the Co-operative Research Project on Biological Resource Management, a four-year endeavour that involves creating fellowships to encourage exchange among scientists in the field, and the organisation of workshops and publications on the proceedings. For more information, please contact the OECD – Directorate for Food, Agriculture and Fisheries, 2, rue André Pascal, 75775 Paris Cedex 16, France.

Opening address

Ladies and gentlemen,

It is a great pleasure and honour for me to welcome you today on behalf of Wageningen Agricultural University. Together with the research institutes of the ministry of Agriculture, Natural Resource Management and Fisheries, our University has made the small town of Wageningen known in many parts of the world as a centre for agricultural research. Ironically, this has not only contributed to a reputation of some degree. Today, agricultural research is under severe attack. A production-oriented approach in agricultural science has been, and still is, successful in leading to the production of abundant, cheap food of good quality. Current production methods, however, have also led to food surpluses in large parts of the industrialized world, to inefficient use of resources, and to environmental pollution and health hazards. In contrast, adequate technology for sufficient food production in less-endowed countries is not yet provided for to an acceptable degree.

Although the problematic nature of these issues should not be denied, they present at the same time challenges and responsibilities for the scientific community to work on. A major shift is now occurring within the agricultural sciences from a production-oriented to an environmental safety-oriented approach. Sustainable land use and sustainable agriculture are key issues in this respect. They require research on efficient use of external inputs wherever these are scarce, such as in large parts of the developing countries, and wherever outputs should be reduced, such as in some parts of the industrialized world. Efficient use of resources is easier said than done. It calls for a lot of basic knowledge on the functioning of natural and managed ecosystems both from a production and an environmental point of view. Soil research is of prime importance in this context, first because supply of water and nutrients depend mainly on soil factors, and second because sooner or later human activities aboveground have repercussions belowground.

Soil research has always been one of the strengths of Wageningen but, until recently, the biological aspects have received relatively little attention. Truly interdisciplinary and basic research is now needed, involving soil biologists, physicists, chemists and plant scientists to unravel the functioning of soils as ecosystems, a prerequisite for an operational approach towards sustainable

land use and agriculture. Such basic soils research is at the heart of the mandate of Wageningen Agricultural University and an important reason to sponsor your workshop.

I wish you every success over the next few days and I hereby declare this workshop open.

H.C. VAN DER PLAS
Rector Magnificus
Wageningen Agricultural University

Methods for the study of interrelationships between micro-organisms and soil structure

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ABSTRACT

Darbyshire, J.F., Chapman, S.J., Cheshire, M.V., Gault, J.H., McHardy, W.J., Paterson, E. and Vaughan, D., 1993. Methods for the study of interrelationships between micro-organisms and soil structure. In: L. Brussaard and M.J. Kooistra (Editors), *Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships*. *Geoderma*, 56: 3–23.

Common methods of assessing soil structure that could be used by microbiologists are reviewed and involve size fractionation of soil aggregates, water release characteristics, intrusion porosimetry, gas adsorption and microscopic methods with image analysis. These methods could also be used for studying aspects of the interactions between soil structure and larger fauna or plant roots. Some recent refinements of these methods are described and the use of artificial soil aggregates in microbial studies is discussed.

INTRODUCTION

Soil structure was defined by Dexter (1988) as “the spatial heterogeneity of different components or properties of soil...”. His definition includes many aspects of structure that are only manifest when soil is considered at different degrees of organization and scale. This wide range of scale often leads to hierarchical approaches to soil structure, in which distinctive combinations of physical, chemical and biological processes are thought to occur at each scale (Tisdall and Oades, 1982). Recent developments in the application of fractal geometry (Mandelbrot, 1982) to describe soil structure (Bartoli et al., 1991; Young and Crawford, 1991) may alter the requirement for arbitrary hierarchies of structure and substitute scale ranges inherent in the data. At present, it is too early to judge to what degree such fractal concepts increase our understanding in addition to improving our characterization of soil structure.

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In this discussion, soil structure is considered mainly at the aggregate/microaggregate scale, as defined by Dexter (1988).

In the early stages of development of soil microbiology, there was often a tendency to regard soils as a rich medium densely colonized with micro-organisms, but direct observations of the soil fabric with light microscopes (Kubišna, 1938) and particularly with electron microscopes (Gray, 1967; Kilbertus, 1980; Foster, 1988) showed that micro-organisms were usually discontinuously distributed throughout soil even in the rhizosphere (Rovira et al., 1974). Some correlation of such microbial discontinuities with structural features has been attempted, notably by Hattori (1973, 1988). He suggested, mainly on the basis of his soil aggregate washing technique, that different microbial communities colonized the outside and inside of soil aggregates.

Differences between the decay of soil organic components in liquid culture and soil (Sørensen and Paul, 1971) have been explained partly in terms of physical separation of organic matter from organisms in soil. The bursts in microbial activity and organic matter decay that follow natural or human disruptions of the soil (e.g. rewetting of soil or cultivations) have also been explained partly in terms of rearrangements of the soil (Rovira and Greacen, 1957; Birch, 1960; Powlson, 1980). Several computer models (Van Veen and Paul, 1981) aimed at simulating organic matter decomposition in soils have assumed that a fraction of the organic matter is physically protected from micro-organisms. Differences in soil structure have thus been used frequently in plausible explanations of soil microbial ecology.

Several methods exist for the characterization of soil structure in the field and laboratory (Burke et al., 1986). This review is restricted to those methods that are most likely to be used by soil microbiologists, including size fractionation of soil aggregates, characterization of the pore space using either water release characteristics, intrusion porosimetry or gas adsorption methods and examination of structure by microscopic methods with image analysis. In addition, the use of artificial soil aggregates in microbiology is discussed.

SIZE FRACTIONATION OF SOIL AGGREGATES

Size distributions of primary particles after dispersion are commonly used to characterize the texture of soils under investigation. Frequently, little further use is made of these analyses in subsequent discussions, but in some instances the distribution of either biomass and microbially-synthesized material (Amato and Ladd, 1980; Cheshire and Mundie, 1981; Ahmed and Oades, 1984; Williams et al., 1987) or the relative proportion of labile and recalcitrant organic matter (Turchenek and Oades, 1979; Bracewell et al., 1980; Elliott, 1986) in the different size fractions of soil have been determined. If the objective is to isolate pre-existing micro-structures from soil and study the

distribution of micro-organisms or organic matter, then minimal dispersion techniques are to be preferred to vigorous mechanical treatments. Ahmed and Oades (1984) also concluded that vigorous shaking of soil slurries with a wrist action shaker destroyed a major part of the microbial biomass, as estimated by ATP content, and smeared the cell contents across the colloidal fractions.

Even rapid wetting can disrupt air-dry aggregates either through compression of air within the porous structure or differential swelling (Koenigs, 1972). In Fig. 1, the size distribution of fragments produced by rapid wetting of 2–5 mm diameter air-dry aggregates is compared with those released by rapid wetting of the evacuated aggregates (E. Paterson, pers. commun., 1991). The removal of air from pores by evacuation enhanced the stability of the aggregates. After such mild disaggregation most of the clay fraction remained bound within the aggregates. Concave surfaces, apparently composed of oriented clay particles, were commonly observed with a scanning electron microscope (SEM) on soil fragments produced by wetting air-dry aggregates without evacuation and suggested that the ruptures occurred along planes of weakness bounded by soil pores (Fig. 2) (E. Paterson and W.J. McHardy, pers. commun., 1991). Dickson et al. (1991) compared the aggregate size distribution after four different wetting treatments and showed that slow wetting at atmospheric pressure usually produced as many water stable aggregates as fast wetting under vacuum. Slow wetting was more convenient in practice. Kemper and Rosenau (1986) reached similar conclusions about the rate of wetting. Dexter (1988) has also suggested that the wet-sieving technique of Kemper and Koch (1966) where the soil is wetted slowly to saturation in a stream of water vapour before it is immersed in water may be appropriate for extremely fragile soils, which would completely slake if wetted rapidly. Prelim-

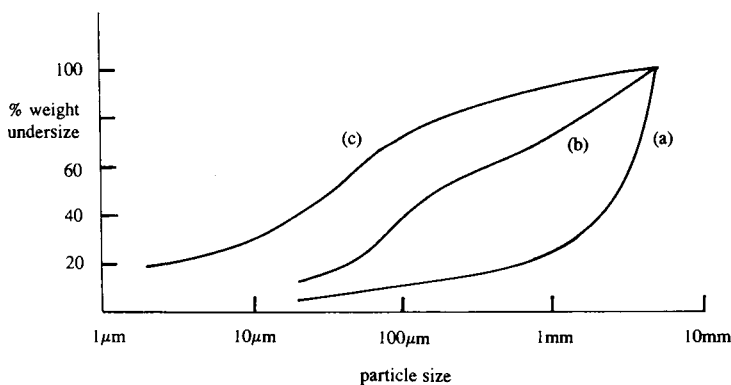


Fig. 1. Size distribution of fragments produced from soil aggregates (2–5 mm diameter) after (a) rapid wetting under vacuum, (b) rapid wetting at atmospheric pressure, and (c) full dispersion by ultrasonic treatment.

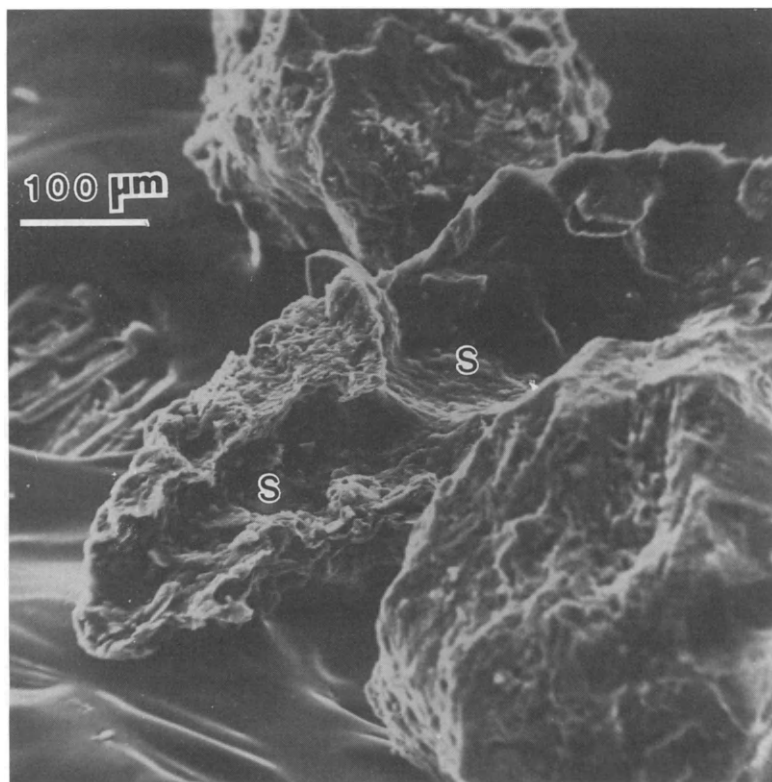


Fig. 2. Scanning electron micrograph of air-dry aggregate after disruption by rapid wetting showing concave surfaces (s) with orientated layers of clay. Secondary electron image.

inary slow wetting of soil aggregates before wet sieving would certainly seem advisable for microbiological studies. Wet sieving machines minimize variation due to different operators by closely controlling the movements of the sieve during sieving (Bartoli et al., 1991, Dickson et al., 1991).

Elliott (1986) used wet-sieving of soil aggregates after vapour wetting or air drying to compare the relative water stability of soil aggregates from native grassland and cultivated soils in the USA. Gupta and Germida (1988) avoided wet-sieving altogether and sieved field moist soil samples in Canada into 5 classes of aggregates between > 1 mm and < 0.1 mm diameter without any water amendments. In agreement with the results obtained with preliminary vapour wetting by Elliott (1986), they concluded that the macroaggregates (> 250 μm diam.) contained most of the carbon and nitrogen and that the rates of mineralization of these elements were greater in macroaggregates than in microaggregates. One disadvantage of sieving field-moist soil samples is that the finer sieves would quickly become blocked and this would limit the quantity of soil aggregates that could be produced by this procedure in prac-

tice. Any differences in field moisture between samples would also be confounded with textural differences between samples.

A recent report of the distribution of microbial biomass in different soil fractions of an Australian Vertisol using a gentle dispersion technique (Bruckert, 1982), which it is claimed disrupts macroaggregates and not the more tightly bonded water-stable microaggregates, is a promising development (Ladd et al., 1990; Jocteur Monrozier et al., 1991). Even this method involves the abrasive action of agate balls. Clearly, whatever minimal dispersion technique is chosen, it should be standardized and described in detail (Oades, 1987). Some means of distinguishing soil fragments from different dispersal methods may help to distinguish the most likely pre-existing microstructures. As fractal geometry has been used successfully to judge the effectiveness of different methods of crushing mineral ores (Kaye, 1989), a similar approach with soil fragments warrants detailed investigation.

Instead of sieving, Postma et al. (1989) separated soil particles into 3 classes ($> 50 \mu\text{m}$, 50 to $2 \mu\text{m}$, $< 2 \mu\text{m}$ diam.) by sedimentation and the application of Stoke's law, assuming that all the particles had a density of 2.65 g/cm^3 . Unfortunately, as there is not a constant relationship between particle size and density, their class boundaries would be liable to some approximation. Small samples of soil were suspended in demineralized water and lightly shaken for 3 min before the suspensions were allowed to sediment. Using this method, they showed that root-nodule bacteria (*Rhizobium leguminosarum* biovar *trifolii*) inoculated into soil samples with small initial moisture contents and probably with only the narrowest pores filled with water, survived better than when larger initial moisture contents were used. The rhizobia appeared to be mainly associated with the largest class of soil particles. Using the same methods in later studies, they showed that the presence of competitive soil bacteria hindered rhizobial colonization of the larger aggregates. Soil flagellates reduced rhizobial numbers associated with the smallest aggregates (Postma et al., 1990).

WATER RELEASE CHARACTERISTIC

The drying limb of the soil water retention characteristic is often used to estimate the pore size distribution in soil samples by assuming that soil pores consist of a series of interconnected cylinders and using the equation $d = 300/Y_m$ relating the diameter d (μm) of largest soil pores filled with soil solution to a given matric potential (Y) (kPa) (Hillel, 1971). Such assumptions are oversimplifications of the soil pore network and are particularly unsuitable for clay soils, which are liable to shrink under suction and cause some rearrangement of the soil particles (Newman and Thomasson, 1979; Smiles, 1988). The moisture retention characteristic is therefore only a general indicator of pore size distribution and is usually applied to soil pores smaller than

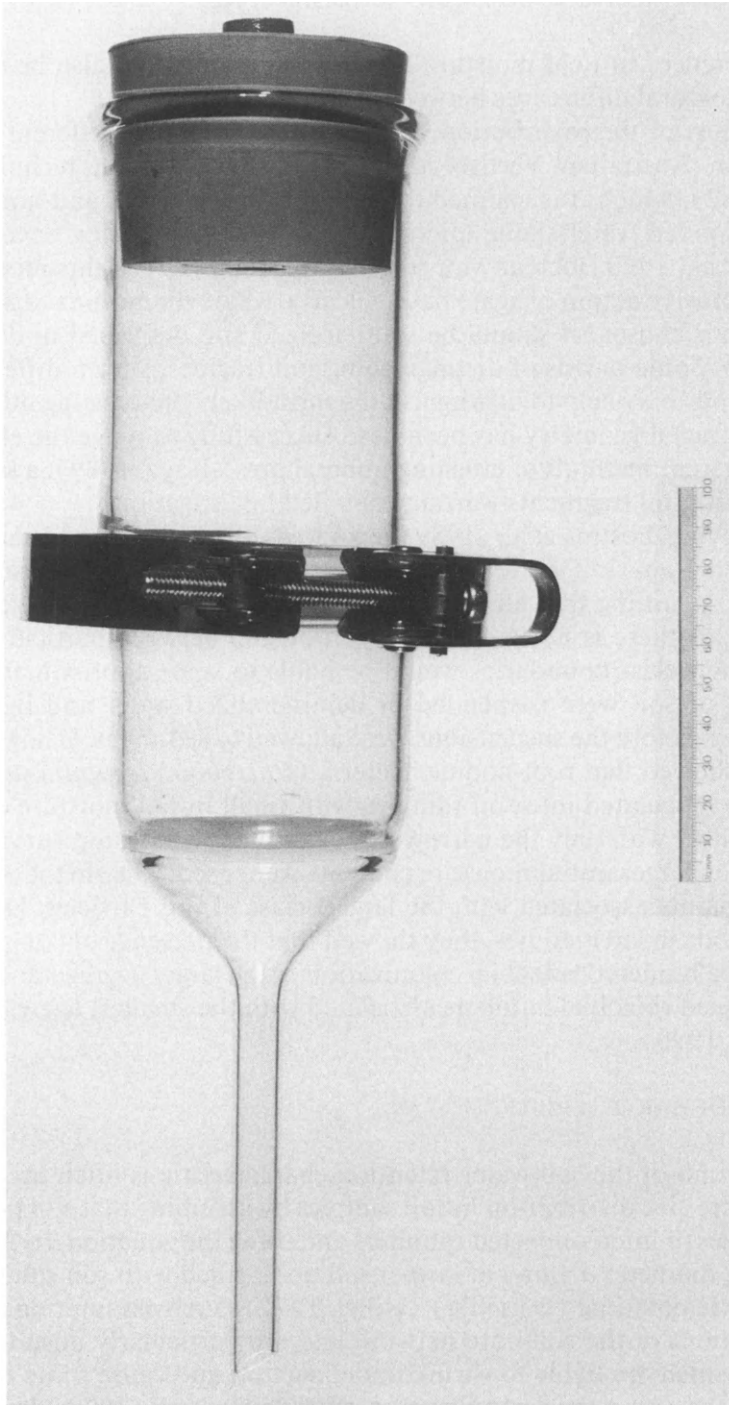


Fig. 3. Glass vessel used with electrolytic respirometer (Tribe and Maynard, 1988) to measure respiration of soil cores maintained at predetermined matric potentials below -10 kPa. Stainless steel clip in centre of vessel, holding 2 ground glass flanges together, can be opened to introduce soil core on top of sintered glass filter (Porosity 4, Gallenkamp) at base of vessel. Scale = 100 mm.

300 μm diameter. Usually, it is determined by measuring the moisture contents of soil samples equilibrated at a range of potentials. The different methods and equipment, e.g. suction tables and pressure plates, used for these determinations have been summarized by Reeve and Carter (1991).

Griffin (1972, 1981b) discussed in detail the significance of moisture release characteristics to microbial ecology, particularly with regard to soil fungi and bacteria. Wallace (1956, 1958a, b) studied the implications of these characteristics for nematodes and more recently these considerations were extended to protozoans (Darbyshire, 1976; Elliott et al., 1980; Vargas and Hattori, 1986; Kuikman et al., 1989; Heijnen et al., 1990; Postma et al., 1990). It was assumed in these investigations that the soil samples were adequately equilibrated at the required matric potential before any sampling occurred and that the microbial inoculants were uniformly distributed, but this latter assumption was not confirmed by direct observations. Recently at the Macaulay Institute, an electrolytic soil respirometer (Tribe and Maynard, 1988) linked to a BBC microcomputer has been adapted to measure microbial respiration of undisturbed soil cores held at predetermined matric potentials below -10 kPa using the glass vessel shown in Fig. 3. At such potentials in most soils, the respiratory quotient is 1 and aerobic respiration predominates (Griffin, 1981a). Such equipment can provide a check that all the soil core replicates within each treatment are respiring uniformly.

INTRUSION POROSIMETRY

Mercury intrusion porosimetry has been used as a rapid method for measuring pore size distributions in soil aggregates (Lawrence, 1977). The method is based on the principle that mercury, a non-wetting liquid on soils and clays, requires an excess pressure to enter a porous matrix. The relationship between the pressure and the pore radius intruded is given by the Washburn equation, which is similar to that used in the calculation of pore size distributions from the water retention characteristics curve. For the pressure range between 1 and 2500 kPa, the pore radius intruded varies from 7.5 μm to 3.5 nm. By measuring intrusion at sub-atmospheric pressures the size range can be extended to include larger pores of up to approximately 75 μm radius (Bartoli et al., 1991). Usually, the experimental results of intruded volume versus pressure are converted to a pore-radius distribution by assuming either a cylindrical (Lawrence, 1977) or a slit-shaped pore (Sills et al., 1973). The same experimental data can also be interpreted using fractal concepts without assuming Euclidean shapes (Kaye, 1989). A detailed discussion of experimental and theoretical considerations is given by Gregg and Sing (1982).

A major advantage of mercury intrusion porosimetry over water release methods for fine textured soils is that water is removed from the soil before and not during the measurement procedure. A wide range of drying methods

may be used with mercury intrusion porosimetry, involving either air drying, freeze drying or critical-point drying (Greene-Kelly, 1973). The actual drying method chosen for each sample will depend on which method induces the least shrinkage and which avoids artefacts in the pore size distributions of the dried sample, such as the ability of the dried sample to withstand mechanical damage during mercury intrusion (Lawrence, 1978; Lawrence et al., 1979; Newman and Thomasson, 1979; Murray and Quirk, 1980). In our experience, Lawrence et al. (1981) appositely summarized the advantages of mercury intrusion porosimetry by stating that "...Critical point drying and mercury injection is the least unsatisfactory procedure available to study pore size distributions in clay soils...." Mercury porosimetry has not been used in microbiological studies.

GAS ADSORPTION

Low-temperature gas adsorption can give information on both the specific surface area and porosity of the solid phase. The specific surface area of a soil can be determined by applying the Brunauer, Emmett and Teller (BET) equation to adsorption isotherm data at low relative pressures. Theoretically, the method can be applied to many sorbates, but in soils it has been restricted largely to water vapour adsorption at room temperature (Newman, 1983) and low-temperature nitrogen adsorption at 77°K (Greenland and Mott, 1978). The size distribution of very small pores ranging from molecular dimensions to 15 nm radius can also be determined from adsorption data using the Kelvin equation. In this case the complete adsorption/desorption isotherm is used and the pore size distribution is calculated from the desorption curve, as in water retention calculations (Gregg and Sing, 1982). Using this approach, the specific surface area and microporosity of allophanic soil clays have been measured (Paterson, 1977) and the results help to explain the accumulation and stabilization of organic matter in Andosols.

Although the pores studied by gas adsorption are very small, the majority of the surface area in many soils is contained within such pores and is unavailable for microbial colonization. This method could be used in conjunction with mercury porosimetry and the water release characteristic to give a nearly complete pore size distribution available for microbial colonization and non-microbial sorption. Few soil microbiologists have characterized their soil samples in these terms.

MICROSCOPIC METHODS AND IMAGE ANALYSIS

Visible and UV light microscopes have been used frequently to describe soil microstructure in thin sections or small slices of soil impregnated with a polyester or epoxy resin (Altemüller, 1974; Bascomb and Bullock, 1974; Fi-

tzpatrick, 1984; Murphy, 1986). The soil samples are often dehydrated with a graded series of mixtures of an organic solvent (e.g. acetone) and water before impregnation with resin. When roots or micro-organisms are present, the soil samples are usually fixed with glutaraldehyde before dehydration (Darbyshire et al., 1985; Tippkötter et al., 1986; Postma and Altemüller, 1990). Porosity can be determined by point-counting (Dexter, 1976), but image analysis with either light microscopy or macrophotography is the principal method used for quantifying microstructure in these embedded samples for soil pores larger than 60 μm diameter (Jongerius et al., 1972; Murphy et al., 1977; Ringrose-Voase and Bullock, 1984). The thickness of the soil thin section limits the smallest pores that can be reliably measured in transmitted light and a common minimal thickness for soil thin sections is about 25 μm . Incident fluorescence microscopy is also restricted by the subsurface fluorescence in the pores when used with soil slices and fluorescent dyes in the resin. Alexander and Jackson (1954, 1955) were first to demonstrate fungi, bacteria and algae in soil thin sections. Later investigations have extended the range of small organisms to include mites, nematodes and protozoa. Recently, the soil thin section technique has been used to study the distribution of fluorescently-stained bacteria in soil pores of different sizes (Postma and Altemüller, 1990).

Autoradiography of ^{14}C -labelled plant material in soil slices has also been used in unpublished microbiological experiments at the Macaulay Institute. The details of the method are described in the Appendix. Table 1 illustrates the type of data that can be obtained from such autoradiographs; from these results nitrogen and phosphorus amendments to the soil appear to have accelerated ^{14}C plant decay. Such autoradiographs may be particularly useful in following root decay and the formation of biopores derived from roots la-

TABLE 1

Effect of nitrogen and phosphorus amendments on the decomposition of ^{14}C -labelled ryegrass (*Lolium perenne* L.) in nutrient-poor soil, (Countesswells Soil Association and series, Glentworth and Muir, 1963), as estimated by autoradiography of soil slices. ^{14}C residues expressed as units of area of grass equivalents $\log_{10}$ (μm^2)

Phosphorus	Nitrogen	
	– N	+ N
– P	4.556	4.001
+ P	4.076	4.051

Results are for ryegrass roots and shoots buried in a layer 1 cm below the soil surface for 24 weeks at 10°C. Soil slices were prepared and analyzed as indicated in the Appendix. Area of grass equivalents were log transformed to normalize the data. Each figure in the table is the mean of nine sections (three sections from each of three pots). LSD ($P < 0.01$) = 0.444.

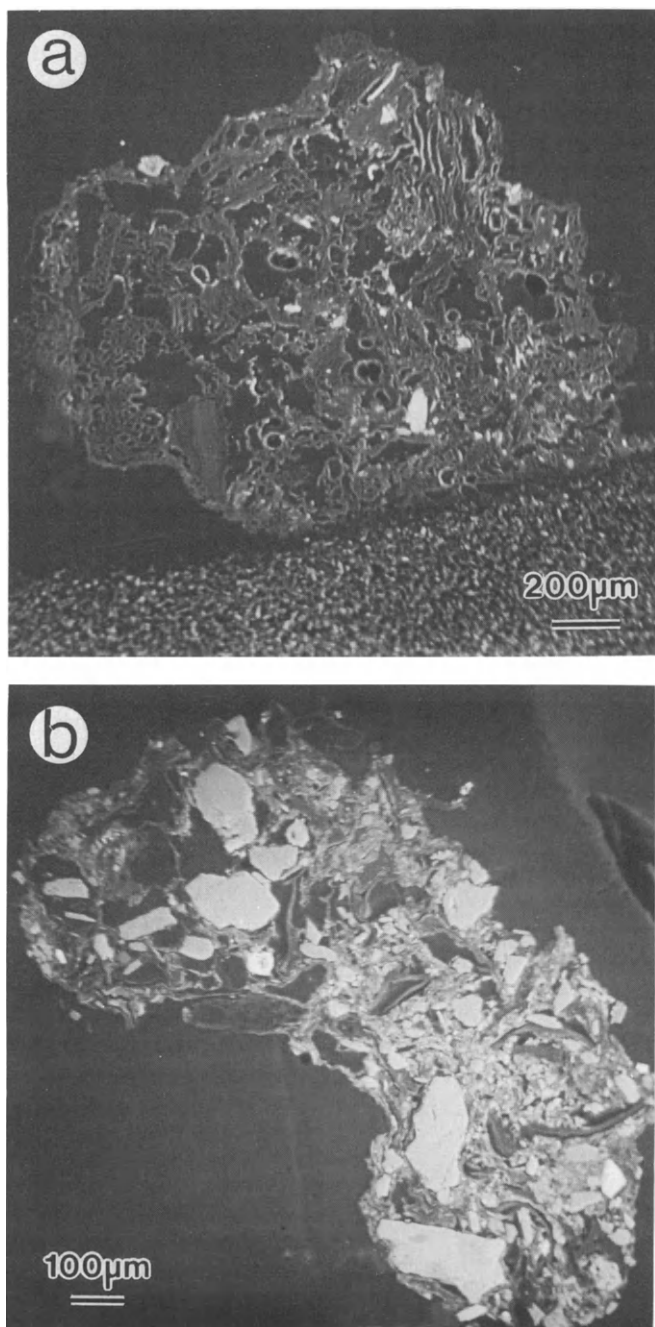


Fig. 4. Scanning electron micrographs of faecal pellets from (a) the Millepede *Glomeris hexasticha* Brandt (Diplopoda) and (b) the wood louse, *Oniscus asellus* L. (Isopoda). Back scattered electron scanning images.

belled in situ with ^{14}C and at a stage when the plant material has become amorphous or difficult to identify. Grossbard (1969) successfully used a similar autoradiographic technique to study the decomposition of ^{14}C -labelled fragments of grasses buried in soil, but without resin embedding. Qureshi et al. (1978) also suggested that autoradiography of soil thin sections was a promising technique.

Low magnification scanning electron microscopy ($\times 100$ to 250) using backscattered electron scanning images (BESI) coupled with image analysis is probably the most promising method for studying porosity below $60\text{ }\mu\text{m}$ pore diameter (Bisdorf and Thiel, 1981; Chretien and Bisdorf, 1983; Darbyshire et al., 1985; Fies and Bruand, 1990). Such images from resin impregnated and polished soil surfaces can provide high contrast between the soil pores and the matrix. Fies and Bruand (1990) investigated the porosity of soil aggregates (2–3 mm diam.) and found reasonable agreement between porosity estimates made by pore volume balance estimation, mercury porosimetry and BESI with image analysis. A comparison between pore size distributions estimated by image analysis of BESI and porosimetry, however, was not presented. Darbyshire et al. (1989) studied the pore size distribution in soil aggregates (2–5 mm diam.) by image analysis of montages (40–50 prints) of BESI. They found that the porosity and pore size distribution showed appreciable variation within a sample of 9 aggregates from the same soil horizon. Later, Glasbey et al. (1991) digitized three of the same montages to produce binary images representing the soil pores and matrix. They also simulated these images in 2 dimensions using a Boolean stochastic model. The same Boolean model was used to create three-dimensional reconstructions of the pore network of the aggregates and to estimate the degree of connectivity between the intra-aggregate pores and the exterior of the aggregates. Running the same model with different aggregate porosities showed that increasing aggregate porosity led to increasing degrees of connectivity between the inside of the aggregate and the exterior. Further investigations are required to test to what extent this relationship between porosity and pore connectivity can be applied to other soil aggregates. Aggregate porosity may prove to be a satisfactory index of the degree of separation between soil organic matter inside the aggregate and the majority of the decomposer soil micro-organisms on the outside of the aggregate. In addition, aggregate porosity may indicate the degree of protection of intra-aggregate microfloral prey from small microfauna, such as protozoa. The porosity of invertebrate faecal pellets may similarly be an important characteristic influencing the relative persistence of organic matter in faecal pellets of different animals apart from any differences in chemical recalcitrance (Fig. 4).

ARTIFICIAL SOIL AGGREGATES

Many investigators have attempted to avoid the problems associated with the inherent heterogeneity of soils by studying soil micro-organisms in alter-

native media such as sand, glass beads, vermiculite, etc. with a restricted range of micro-organisms. Another approach has been to amend soil samples with artificial aggregates or biological remains and study the microbial activity in the immediate vicinity of these amendments. Many of these studies with artificial soil aggregates have been concerned more with aggregate stability or decomposition of fertilizers than with microbial communities per se (Chandra et al., 1962; Harris et al., 1963). Webley and Duff (1962), however, described a method for studying localized microbial activity by incorporating soluble and insoluble organic compounds in compressed kaolin pellets, which were then added to soil samples. This technique was later extended to include microbial residues (Webley and Jones, 1968; Jones and Webley, 1968). With this technique, it was possible to correlate biochemical transformations of the amendments in and around the pellets with the succession of microbial colonists. Because of the compressed nature of the pellets, however, most of the microbial activity was confined to their outer surfaces and the pellets did not resemble the structure or mineralogy of natural soil aggregates. Recently, at the Macaulay Institute, the production of artificial aggregates with pore systems more analogous to those found in natural soil aggregates has been investigated (E. Paterson, pers. commun., 1991). They were prepared by remolding the B horizon clay soil from the Peterhead Soil series (in wt%: sand 41, silt 20, clay 39, Glentworth and Muir, 1963) at a moisture content close to

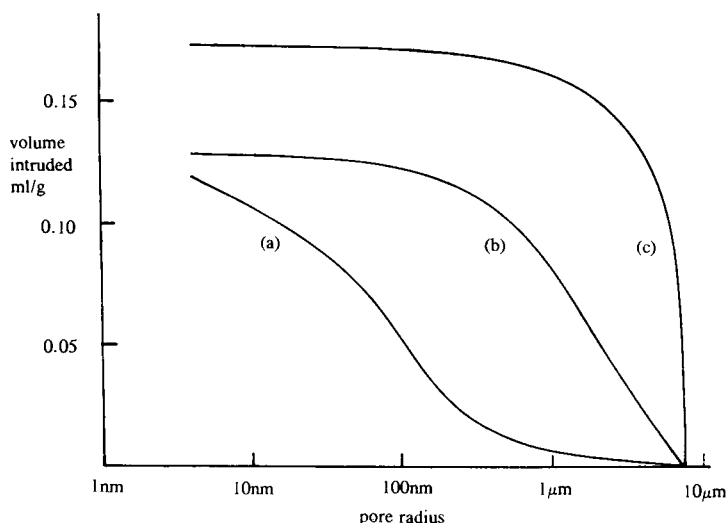


Fig. 5. Pore size distributions obtained by mercury intrusion porosimetry for (a) original air-dried soil aggregates (2–5 mm), (b) oven-dried artificial soil aggregates, and (c) freeze-dried artificial soil aggregates.

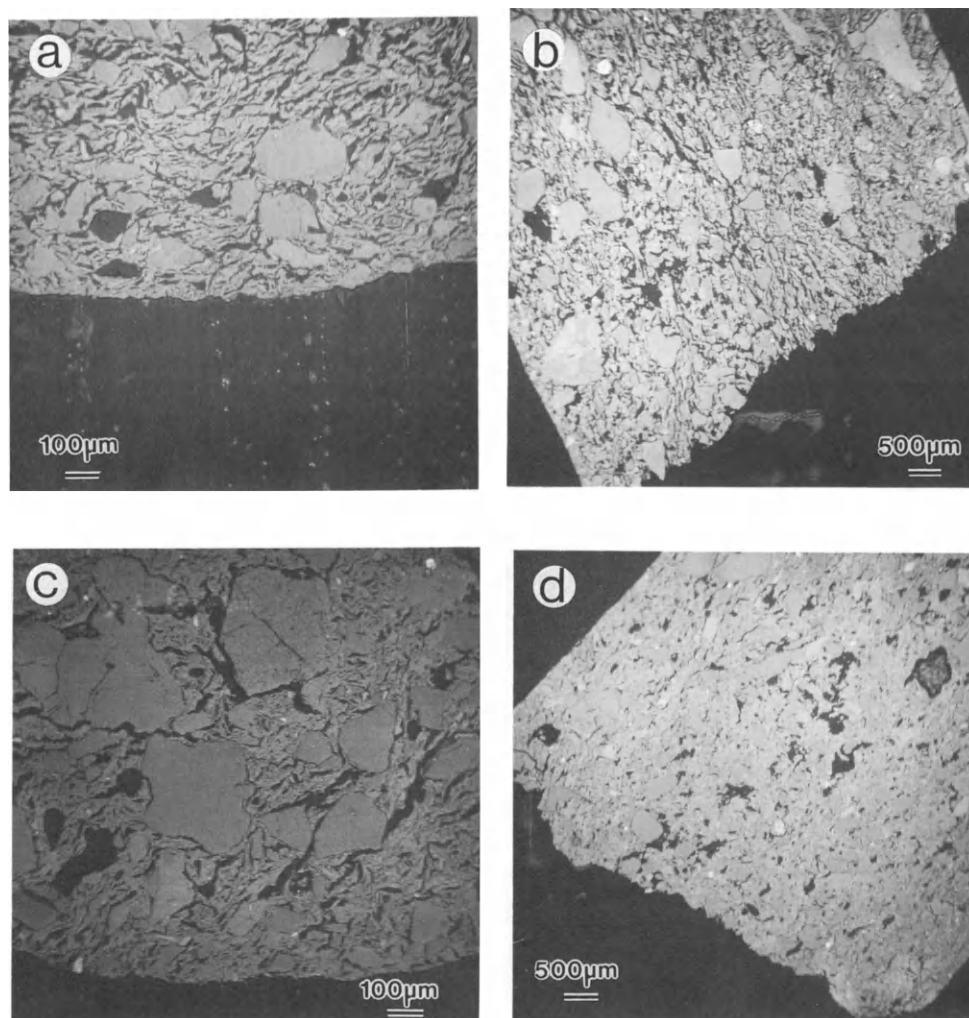


Fig. 6. Scanning electron micrographs of artificial soil aggregates in the form of cylinders. Longitudinal sections (b) and (d), transverse sections (a) and (c). Freeze-dried aggregates (a) and (b); oven-dried aggregates (c) and (d). Back scattered electron scanning images.

the plastic limit (in wt%: liquid limit 48.6, plastic limit 19.0). A 50 g sample of air-dry < 5 mm soil was mixed with 10 ml of distilled water until a homogeneous stiff paste was obtained. It was then divided into six equal parts that were rolled on a glass plate to produce six cylinders 4–5 mm in diameter. Three of these cylinders were then dried overnight at 105°C and the oven-dry moisture content calculated. The other three were immersed in liquid nitrogen and freeze dried. After drying, the two sets of cylinders were broken into short segments (5–8 mm) and ignited in a muffle furnace at 1000°C for 24 h to increase their mechanical strength. The porosities of these ignited mate-

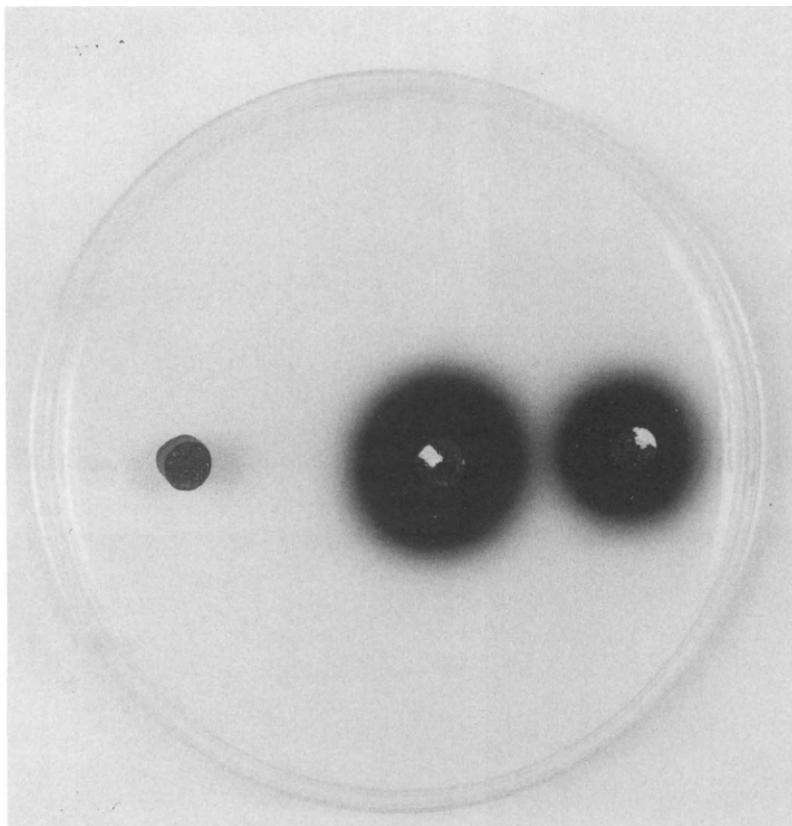


Fig. 7. Artificial soil aggregates incubated for 18 h at 20°C in petri dishes (11 cm diam.) containing agar (1.2% w/v), urea (0.5M) and bromocresol purple (0.01% w/v). Central aggregate was immersed in purified urease solution (50 $\mu\text{g}/\text{ml}$) for 18 h at 4°C, rinsed in distilled water before incubation on agar. Note the halo around this aggregate due to diffusion of urease from aggregate, release of NH_3 from urea substrate and colour change of bromocresol purple. The aggregate on the right was similarly treated with urease, but was buried in non-sterile soil for 5 days prior to incubation on agar. The aggregate on the left was recently ignited in muffle furnace and contained no urease.

rials were then compared using mercury intrusion porosimetry with that of the 2–5 mm aggregates isolated by dry-sieving the original soil (Fig. 5a). The total intruded volume for the air-dried soil aggregates was 0.12 ml/g and 90% of the pores lay in the size range 4–400 nm. The oven-dried, remolded soil after ignition had a similar intruded volume, but the size distribution was markedly different with most of the pores in the size range 400 nm to 6 μm (Fig. 5b). The most striking change could be seen for the freeze-dried remolded soil after ignition where the intruded volume was 50% greater at 0.17 ml/g and the size distribution much narrower, presumably due to the nucleation of ice crystals during immersion in liquid nitrogen (Figs. 5c and 6). Low-

temperature nitrogen adsorption measurements showed that after ignition the artificial aggregates had very low values of specific surface area ($< 1 \text{ m}^2 \text{ g}^{-1}$) compared to the original soil ($23 \text{ m}^2 \text{ g}^{-1}$), presumably due to sintering of fine particles. This reduction in specific surface area will be accompanied by a significant reduction in surface reactivity and should enable surface-induced effects to be distinguished from pore size effects.

These preliminary results suggest that a range of porosities and pore size distributions could be created in artificial soil aggregates by either varying the water content during remolding or by altering the texture of the soil sample before remolding by addition of extra clay size material previously separated from the original soil. Micro-organisms or organic compounds can be incorporated inside such aggregates by soaking them in solutions containing either micro-organisms or organic compounds prior to their addition to soil. Autoradiography could also be used, if any of the micro-organisms or compounds were radiolabelled. Microscopy could be employed to observe micro-organisms in situ. Figure 7 shows the results of an enzyme assay with such soil aggregates for studying the persistence of urease in soil (D. Vaughan, pers. commun., 1991). In this assay, the aggregates are first immersed in purified urease solutions ($50 \mu\text{g/ml}$) for 18 h at 4°C and then rinsed in distilled water before they are incubated either in soil, autoclaved vermiculite or water for up to 8 days. Urea (0.5M) and the pH indicator dye, bromocresol purple ($0.01\% \text{ w/v}$), are incorporated into agar ($1.2\% \text{ w/v}$) within petri dishes. Subsequently, some of the soil aggregates are tested for urease activity at daily intervals by incubating the aggregates with their porous ends in contact with the agar for 18 h at 20°C . During this incubation, urease diffuses from the ends of the soil aggregates, NH_3 is released from the urea substrate and a colour change from yellow to red develops in the agar near the soil aggregates.

Apart from use in theoretical studies of microbial ecology, such artificial soil aggregates could be used in more practical subjects, e.g. in improving the effectiveness of soil microbial inoculants.

CONCLUSIONS

Several methods now exist for the characterization of soil structure at the aggregate/microaggregate level and microbiologists should try to ensure that the physical conditions of the soils used in their studies are defined by these methods. Most investigations will be restricted by the equipment available locally, but it is advisable to use a combination of methods to characterize soil structure wherever possible, e.g. water release characteristics and microscopic methods. The use of artificial soil aggregates should promote understanding of the relative importance of different pore sizes to micro-organisms and help to distinguish between surface reactivity and pore size effects. New methods should also be fostered and existing methods further refined. Such

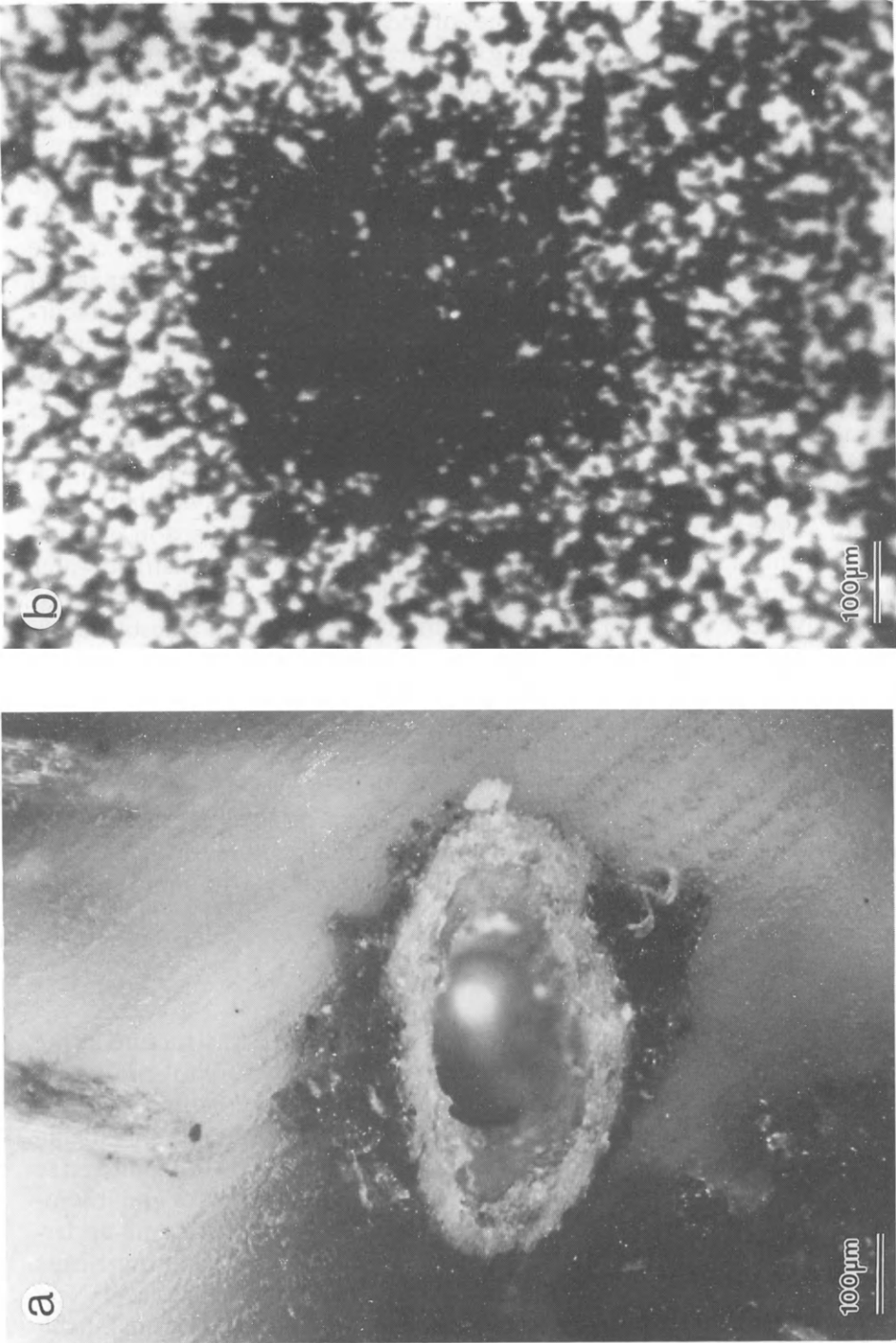


Fig. 8. (a) ^{14}C -labelled plant fragment of perennial ryegrass (*Lolium perenne* L.) in soil slice embedded in epoxy resin, (b) autoradiograph of same plant fragment. Incident illumination.

refinements should not be restricted to techniques and equipment, but should include new concepts of visualising soil pores. For this reason, the use of fractal geometry to describe soil structure may represent an important development in our attempts to understand the activities of micro-organisms in soils.

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APPENDIX. AUTORADIOGRAPHIC METHOD FOR ^{14}C -LABELLED ROOTS AND SHOOTS OF PERENNIAL RYEGRASS (*Lolium perenne* L.) IN SOIL SLICES EMBEDDED IN EPOXY RESIN

In these experiments, ^{14}C -labelled shoots and roots of perennial ryegrass (*Lolium perenne* L.) were incubated in soil 1 cm below the surface in pots (18 cm max diam.) at 10°C for periods up to 1 year. At intervals, 3 pots were removed from the experiment and soil samples were embedded in resin in Kubiéna cans (Tippkötter et al., 1986). Later, the embedded soil samples were cut into 4 cm thick slices with a diamond tipped saw and one surface of each section polished smooth with 600 grade carborundum powder. The polished face of each section was lightly clamped on to X-ray film (Kodak X-Omat AR) for 2 weeks at room temperature and then the autoradiographs were developed. An autoradiographic ^{14}C -microscale (Amersham International plc., Amersham, UK) was incorporated on to each autoradiograph with 8 grey levels representing levels of activity from 1.15 to 32.67 kBq g^{-1} . Acetate copies of each autoradiograph were made (Copier 5046, Rank Xerox Ltd., Uxbridge, UK). When these acetates were superimposed over each polished soil slice, the position of each radioactive area could be identified and correlated with plant material under a fluorescent microscope with incident illumination (Fig. 8). Image analysis of the autoradiographs was accomplished by transmitted illumination and a black and white video camera linked to an inexpensive PC computer with a digitizing card (Skye Instruments Ltd., Llandrindod Wells, UK). The image analysis program (Skye Instruments) allowed the area of each grey level, corresponding to the activities on the microscale, to be measured in a total area on the autoradiograph of $71.2\text{ mm} \times 42.9\text{ mm}$. The sum of the products of area and activity then gave an indication of the total radioactivity on each autoradiograph ($\mu\text{m}^2 \cdot \text{kBq} \cdot \text{g}^{-1}$). Since different batches of grass used in the experiment varied in specific activity, the above products were divided by the relevant specific activity (kBq g^{-1}) to give values for the ^{14}C residues in units of μm^2 , the "area of grass equivalents". These units should be considered somewhat arbitrary, because it cannot be assumed that the ^{14}C in the grass embedded in the resin would give an identical response to that shown on the microscale.

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Observing soil biota in situ

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ABSTRACT

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Rhizotrons and minirhizotrons allow repetitive, nondestructive observation of the soil biota. Bias associated with minirhizotron and rhizotron observations, and methods that will help realize the potential of these observational platforms are reviewed. Root density estimates in minirhizotrons are prone to bias due to soil compaction, and poor contact between tube and soil. Density estimates of microarthropods observed in minirhizotrons, are less biased by longer observation periods, low light levels, and good optical resolution. Microarthropods observed in a rhizotron were underrepresented compared with soil cores if they belonged to groups that move little, are transparent, or small. Time-lapse video can be used to sample microarthropod density in rhizotrons. Better visualization of all of the biota can be achieved by using long working length microscope objectives, and stains such as fluorescein diacetate, ethidium bromide and the tetrazolium dye, p-Iodonitrotetrazolium Violet. Repeated observation of the same roots allows the use of demographic methods for calculation of root survivorship, turnover, and productivity.

INTRODUCTION

Minirhizotrons and rhizotrons are revolutionizing soil biology by making it possible to nondestructively observe the soil biota in situ. Root growth and physiology have been studied by direct observation in the six lysimeter type rhizotrons in North America (Huck and Taylor, 1982). During the 1980's opportunities for direct observation of the soil biota increased because of the commercial availability of minirhizotron video cameras starting in 1984 (J.M. Bartz, pers. commun., 1991) the construction of an experimental rhizotron at the University of Michigan Biological Station, and the availability of computer-based image analysis systems.

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These new observational tools allow the in situ study of spatio-temporal relationships of the entire range of soil biota: roots, microorganisms, protozoa, and invertebrates. Kubiëna (1955) would have made a stronger case for invertebrate control of humus formation if he could have watched the biota colonize earthworm burrows and collembolan faecal pellets rather than relying on soil sections. Similarly, Hole (1981) could have relied less on descriptive soil surveys to make his argument for animal effects on soil structure if he had had access to direct observation.

Minirhizotrons are relatively easy to install, and thus their increasing use for comparative studies in natural habitats should be expected. Exploration of below-ground habitats with minirhizotrons is important because more than half of net primary productivity is below-ground in the five natural communities for which data is available (Caldwell, 1987): root productivity is greater than previously estimated (Long et al., 1989).

Availability of minirhizotrons and experimental rhizotrons focus attention on sources of bias and inaccuracy in the data that can be collected with them as well as on methodology for their use. Sources of the methodology include methods used for data collection aboveground and laboratory methods that can be adapted for use in situ. The purpose of the present paper is to review biases and new opportunities offered by minirhizotrons and rhizotrons.

ROOTS

Sampling bias

Minirhizotron tubes or rhizotron windows may cause a number of kinds of bias in estimates of root density. Instillation may either compact the soil around minirhizotron tubes, or leave a space between tube or window and soil. For rhizotrons, reconstruction of the soil profile may affect root density, but Atkinson (1985) found no difference between root density of apple trees next to windows at the East Malling rhizotron and bulk soil. For minirhizotrons, a number of methods are used for minimizing soil compaction during installation, and for keeping good contact between soil and tube (Van Noordwijk et al., 1985). In order to tightly fit the minirhizotron tube in soil with a minimum of compaction, Box et al. (1989) suggested pushing tubes into the ground by applying force on the inside bottom in order to stretch and thin the tube when entering the hole. Gijsman et al. (1991) inserted a metal frame into the ground and inflated a rubber tube in it; observations were made with the tube removed and nothing between the lens and the root surface. Soil compaction led to underestimates of root density in some studies (Cheng et al., 1990). Gijsman et al. (1991) commented on the difficulty of making observations during spring and fall due to condensation on the inside of plastic minirhizotron tubes. Observed root density should be compared with density

from core samples for both rhizotrons and minirhizotrons. Vos and Groenwold (1987) suggested that conversion factors based on root density in soil cores be used for minirhizotron data. In spite of the methodology just reviewed, biases caused by instillation of minirhizotrons and rhizotrons in highly structured soil have not been assessed with a variety of methods.

A potential source of bias for rhizotrons is extensive flat surfaces which may make root systems atypical, or change their phenology. Atkinson (1985) showed that root systems of apple trees around the East Malling rhizotron were not atypical in shape or phenology. Further he pointed out that the volume of soil that can be sampled at rhizotron windows may be as great as that in cores, and sampling windows requires less work. The great observational area in rhizotrons allows study of the biological basis of spatial variability of roots.

Minirhizotrons can be installed among crops or in natural communities receiving experimental treatments, and, experimental manipulation next to minirhizotron tubes is possible also. For example, Rush et al. (1984) placed sclerotia of *Phymatotrichum omnivorum* on minirhizotron tubes and allowed cotton seedling roots to grow over them, permitting investigators to photograph mycelial strands proliferating from infected roots. Nutrients or inoculum could be added to the surface of minirhizotron tubes through cannulae. In rhizotrons manipulations may be applied by removing and replacing windows, or by adding materials through holes drilled through them. Experimental manipulations that might be applied to small soil volumes next to minirhizotrons or behind windows include release of CO₂, and addition of known fungal or bacterial inoculum, invertebrates, microspheres, dyes, or small amounts of nutrients. Removal experiments that might be conducted include removal of roots belonging to particular species, clipping or freezing of roots to simulate herbivory, and reduction of invertebrate populations with biocide application.

Root density and productivity

Minirhizotrons have been used to contrast response of roots to different tillage (Cheng et al., 1990), crop (Hansson and Andren, 1987), and community types (Aerts et al., 1989). These studies took advantage of the ease of locating minirhizotrons in the field, but did not take advantage of the opportunity to follow development of individual roots.

The ability to observe nondestructively makes it possible to record development of individual roots of known age in minirhizotrons and rhizotrons. Such data allows application of demographic methods to roots for the first time. Demographic methods are especially helpful in quantifying root turnover and productivity. For example, if a cohort of roots that are the same age is followed through their developmental stages—white, brown, missing, dead—

the age-specific probabilities of entering the next stage or dying can be tabulated and used to calculate survivorship, productivity, and turnover. This is comparable to the cohort life table approach. A second approach, comparable to a static life table, is to start with a population of roots of unknown ages and record developmental changes. These records for individual roots may be used to calculate the probability of the average root going to the next stage, and will give the same estimates of survival, production, and turnover as the cohort approach if there is no long-term environmental change and no change in root density. Hendrick and Pregitzer (1992) made this kind of analysis for roots of a hardwood forest in northern lower Michigan during one year. They summarized changes from white to brown to dead roots in a table of transition probabilities for intervals between each sampling date.

Root productivity measured by recording simultaneous growth and death of cohorts of roots is more accurate than summing statistically significant increases in root biomass plus significant increments in dead root biomass between sampling times. Hendrick and Pregitzer (1992) found a 54% (top 30 cm) and 46% (30–60 cm) higher, and Cheng et al. (1990) a 40% higher root productivity than if they had summed growth and mortality increments. Further, Hendrick and Pregitzer (1992) noted that 95% of the roots disappeared before they were in the dead category. This evidence of rapid decomposition and/or herbivory would have been missed if individual roots had not been followed.

Aids to visualizing roots

Infrared lighting emphasizes contrasts. Dark roots, roots of legumes, cereals, and native grasses are easier to see with UV light. Other ways to visually emphasize roots and their activity include adapting laboratory methods for use in rhizotrons. For example, fluorescein diacetate (FDA) was used by Söderström (1977) to distinguish living from dead fungal hyphae in laboratory preparations. FDA is hydrolyzed to free fluorescein by many enzymes, hence fluorescein is a good indicator of viability. In rhizotrons with removable windows, FDA may be sprayed on soil faces causing metabolically active roots and other biota to fluoresce in situ. Ethidium bromide (EB) is another fluorescent dye that can be used to visualize metabolic activity in eucaryotic cells because it binds with DNA and RNA. Roser (1980) showed that EB stained nuclei of active root, fungal, and bacterial cells. EB could be sprayed on soil in aqueous solution and observed with appropriate filters although, because of its toxicity, EB could not be used repeatedly.

Microorganisms

Macroscopic fungal structures may be counted in rhizotrons and minirhizotrons allowing density estimates of ectomycorrhizae, rhizomorphs, and col-

onies of saprophytic fungi. Periodic observations of the same ectomycorrhizae, and saprophytic fungal colonies allow determination of age-specific survivorship and of productivity using life table methods. Even simple records of growth may be useful when they are related to other biota. In Fig. 1, for example, changes in area of four *Coenococcum geophilum* ectomycorrhizae are negatively associated with numbers of microarthropods observed in the quadrat ($p < 0.01$, Wilcoxon matched-pairs signed-ranks test).

Higher magnifications may be achieved by using long focal length microscope objectives, with the light source off center to enhance contrast (L.E. Casida, pers. commun., 1991). The use of a compound microscope with reflected light to visualize microbes in situ was described by Casida (1969), and confocal microscopes may be useful in this way as well.

Additional laboratory methods that we believe will be useful in rhizotrons are UV light, FDA, and tetrazolium dyes. The length of roots containing vesicular-arbuscular mycorrhizae can be counted non-destructively by using the observation of Ames et al. (1982) that arbuscules in living roots autofluoresce. Application of this method requires use of quartz glass, a concentrated source of UV light, and appropriate filters (e.g. Allen et al., 1989).

FDA is useful in staining active hyphae as well as roots. EB has been used as a counterstain with FDA in laboratory studies of bacteria. EB combines

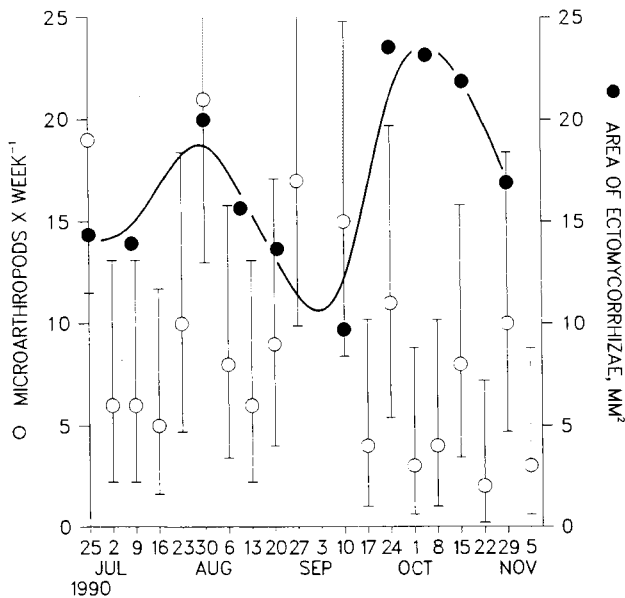


Fig. 1. Total microarthropods observed per week and the area of four *Coenococcum geophilum* ectomycorrhizae in the same 1 cm² area. Observations were made with time-lapse video in the University of Michigan Soil Biotron. The heavy lines are three-week running averages. Vertical bars are 5% Poisson confidence limits around total microarthropods seen per week.

with DNA regardless of viability, staining non-viable bacteria orange in Janagin and Luchsinger's (1980) study, while viable bacteria fluoresced yellow-green from FDA.

Metabolically active microorganisms reduce the tetrazolium salt p-iodonitrotetrazolium violet (INT), producing formazan particles which are easily visible within cells. These dyes were used to quantify metabolic activity of saprophytic fungi (MacDonald, 1980) and external hyphae of vesicular-arbuscular mycorrhizae (VAM) (Sylvia, 1988). Use of INT in a rhizotron would require spraying a solution of INT, NADH, and buffer on the soil face, and observing formazan with a compound microscope.

INVERTEBRATES

Sampling bias

Direct observation of invertebrates in minirhizotrons and rhizotrons is appealing because the methods used to extract invertebrates from soil select mobile, desiccation-resistant species. There is no bias in direct counts of large invertebrates such as earthworms or Scarabaeid beetle larvae in minirhizotrons and rhizotrons unless it is shown that they are affected by plastic or glass surfaces. Carpenter (1988) demonstrated that diplura feed on roots by observation in a rhizotron.

Direct counts of smaller invertebrates such as microarthropods, nematodes, and protozoa, involve biases. Microarthropods will be used to illustrate bias encountered in direct counts using minirhizotrons and rhizotrons.

Minirhizotrons

Snider et al. (1990) used minirhizotrons to observe collembola around roots of corn (*Zea mays*), soybeans (*Glycine max*) and sugarbeets (*Beta vulgaris*) in Michigan. They showed that (1) more than half of the collembola population moved below 30 cm after the middle of August in each crop, and (2) collembola density was correlated with change in root number per time. Snider et al. (1990) also showed that resolution was so poor that only larger, moving collembola could be counted. This effectively limited the study to *Folsomia candida*. They suggested that when invertebrates are filmed in minirhizotrons the camera should be held still long enough so that movements of the animals can be used to aid in identification, the camera focus should be carefully monitored, and condensation on the inside of the tubes in fall and spring should be avoided by filming early or late in the day. Finally, high light levels used in filming in minirhizotrons clearly drives invertebrates away.

Rhizotrons

Litter invertebrates can be observed in the rhizotron described by Carpenter et al. (1985) because the windows extend above the soil surface. Windows are recessed in the University of Michigan Soil Biotron described by Fogel and Lussenhop (1991) making it difficult to focus a dissecting microscope on the litter. With this limitation recognized, data collected at the Soil Biotron was used to compare density estimates made by direct counts through the windows with estimates made by counting microarthropods extracted from soil cores collected next to the windows.

Direct counts of microarthropods were made by observing $1.2 \text{ cm} \times 12.5 \text{ cm}$ strips on the safety glass with a dissecting microscope at 12 to $50\times$. Microarthropods were counted in an area of 3037 cm^2 on August 15, 1989, and an area of 553 cm^2 on May 23, 1991 at depths of 2.5, 6, and 9 cm. It is possible to see about 2.5 mm into the soil, a Rubicon sand; accordingly the area observed was multiplied by 2.5 mm to estimate density per volume. The total volumes sampled were 759 cm^3 , and 138 cm^3 in August and May.

On the same dates, soil cores were collected 0.5 m away from alternate windows of the Soil Biotron. The soil cores were 19.17 cm in diameter and 9 cm deep. The total volumes of soil sampled with soil cores were 2.7 and 8.3 times greater than the volumes observed in August and May respectively.

After collection, cores were divided into 3 cm sections, and fitted into holes in a foam rubber mat over crystalizing dishes of picric acid in a high-gradient extraction apparatus (Lussenhop, 1971). Intensity of heat above the cores was increased and temperature of water circulating below was lowered during a six day period, causing the microarthropods to move down through the drying soil and fall into the picric acid. When the soil core sections were dry, the dishes of picric acid were filtered, microarthropods were removed from the filter paper under a dissecting microscope, mounted in Hoyer's medium, and counted under a compound microscope. This type of extraction method was found, in a thorough survey (Edwards and Fletcher, 1971, table IV), to be significantly more efficient for mobile, desiccation-resistant species such as oribatids, than for less mobile, desiccation-susceptible groups such as collembola, and protura.

Movement, color, and size were key factors in recognition of microarthropods in situ. Only prostigmatid mites (August) and protura (May) were observed in statistically greater numbers than in extractions (Fig. 2). The difference is due to the susceptibility of prostigmatids and protura to desiccation during extraction. An important, though statistically insignificant trend, was for small-bodied, slow-moving groups to be underrepresented by direct observation. This is why astigmatid and oribatid mite density tended to be lower when observed directly than when soil extraction was used. Aggregations of the hypopae of astigmatid mite species and collembola were observed; direct observation offers a method to study this important phenomenon.

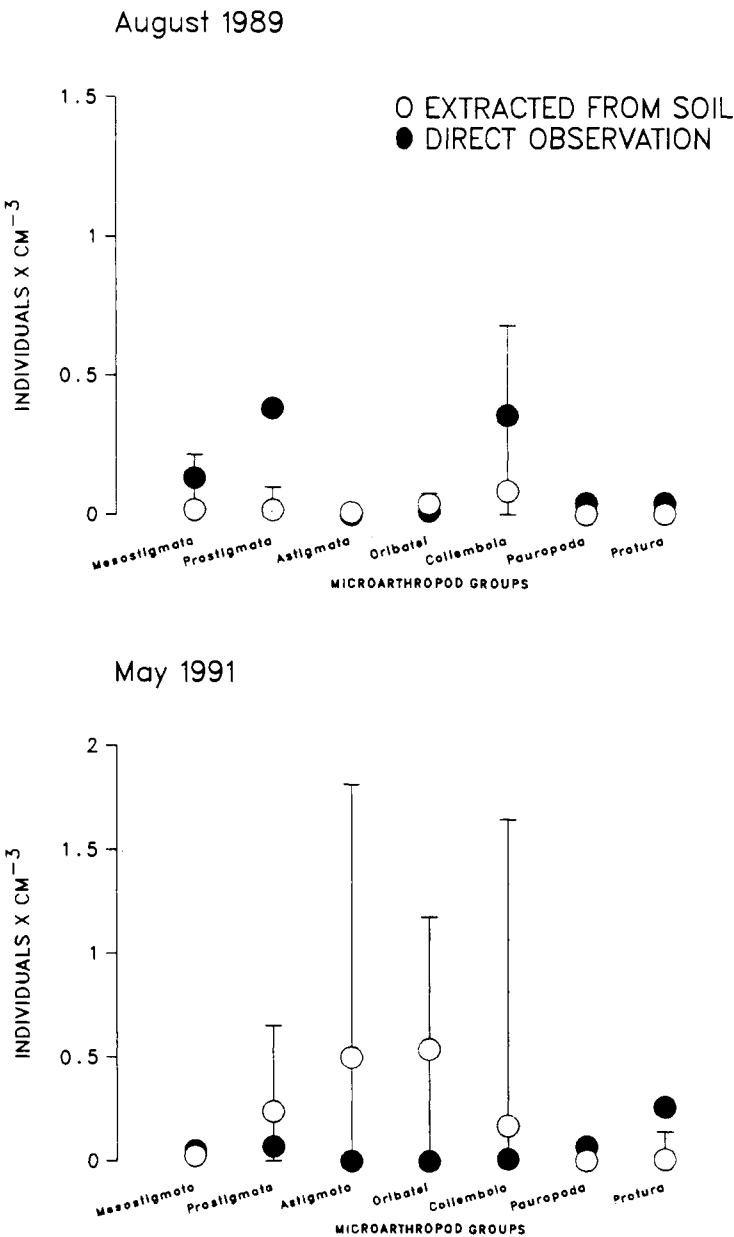


Fig. 2. Comparison of microarthropod density determined by direct observation of rhizotron windows with microarthropods extracted from soil cores collected next to rhizotron windows. Each point is the average of seven 172.5 cm³ cores for soil extraction $\pm$ 5% student-*t* confidence intervals and 759 cm³ and 138 cm³ sampled by direct observation in August 1989 and May 1991, respectively.

Density estimates from time-lapse video

Invertebrates are infrequent in time lapse video recordings because the field of view is small, usually about 1 cm². Overall, an average of 0.003 microarthropods per cm² per 30 second interval were observed between July and November 1990 in the University of Michigan Soil Biotron. Further, microarthropods appear independently of one another. For these reasons the number of microarthropods per unit time fits the Poisson distribution, at least at the spatio-temporal scale of video recording (Table 1). This is useful because it suggests the appropriate tests for confidence limits and comparisons of means. Questions of ecological interest can also be answered using the fit of microarthropod appearances to the Poisson distribution. For example, the length of time a root surface is expected to be occupied by a microarthropod can be calculated.

An example of the use of time-lapse video to estimate microarthropod density is the five month time-lapse record shown in Fig. 1. The number of microarthropods observed per week rose twice, and declined in the fall. On the ten dates when the area of *Coenococcum geophilum* ectomycorrhizae was measured, microarthropod occurrence was negatively correlated with ectomycorrhizal area (Wilcoxon matched-pairs, signed-ranks test, $p < 0.01$).

Interactions among soil biota

Herbivory and other plant–animal interactions are important enough above-ground to justify much space in general ecology texts. It is hard to imagine that interactions of invertebrates with roots, protozoa, and microorganisms is not just as important. The observations of interactions that have been made suggest the potential of in situ observation. For example, Linford (1939) demonstrated that nematodes are attracted to the extension zone of roots by using direct observation of roots growing against glass; time-lapse cinematog-

TABLE 1
Fit of microarthropod numbers in 30 second video records to the Poisson distribution

Number of microarthropods per interval	Frequencies			
	April 16–May 28, 1991		June 25–Nov. 6, 1990	
	Observed	Expected	Observed	Expected
0	912	912.4	1472	1472.8
1	24	23.3	49	47.4
> 1	0	0.3	0	0.8
Total	936		1521	

raphy of the extension zone of apple roots in the East Malling Rhizotron was used to further study this attraction (Pitcher, 1967). The possibility that nematodes reduce contact between fruit tree roots and soil was raised by Atkinson and Wilson's (1979) observations in the East Malling rhizotron. For microarthropods, counts through windows of the University of Michigan Soil Biotron showed a density on tree roots two orders of magnitude greater than in the adjacent soil Lussenhop et al. (1990). With these exceptions, students of soil invertebrates have not used direct observation to study behavior or interactions with other biota.

CONCLUSIONS

In situ observation of the soil and its biota allows repetitive observations of spatio-temporal relationships. Visualization of soil processes will be aided by adaptation of laboratory techniques. Repetitive observation allows improved estimates of root and microbial turnover and productivity, which are relevant to global productivity and carbon balance. In addition, minirhizotrons and rhizotrons allow observation of interactions between the soil biota. Interactions among the soil biota are likely to be at least as important to ecosystem functioning as above-ground interactions such as herbivory are. In short, now that we can see the soil biota in their natural setting, soils will be more a part of the ecosystem.

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Distribution and dynamics of shrub roots in recent coastal dune valley ecosystems of Belgium^α

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ABSTRACT

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A chronosequence, up to 60 years old, was investigated in the Westhoek Nature Reserve. Root growth of the shrubs in dune valleys was found to be restricted to the surface Albi and Bbi horizons which often did not exceed 25 cm. The thickness of these horizons and the amount of litter and humus could be explained by the position along secondary topo-hydrosequences within the chronosequence. The mostly stratified sands underlying the Albi and Bbi horizons had a higher bulk density. In these relatively dense Cd horizons root growth of new colonizing shrubs seems to be possible only along old root or stem channels of the previous vegetation of dune building plants such as *Ammophila* and *Salix* which grew in pace with the accumulating dune.

It is concluded that root growth in these sandy soils is restricted by the high penetration resistance of the closely packed sands in the subsurface horizons. The root penetration problem in these sandy soils explains the uniform vegetation physiognomy better than other climatic or edaphic factors.

INTRODUCTION

In the dune valleys of the Nature Reserve of the Westhoek, shrubs not more than 2 m high dominate from about 20 years of stabilisation till more than 60 years, in spite of identical relief positions and soil moisture conditions throughout the chronosequence.

Previous studies and the traditional approach to soil site morphology description (FAO, 1977; Klinka et al., 1981) provide data that are too vague for a clear understanding of the vegetation distribution in the dune valleys of the Nature Reserve of the Westhoek. New techniques are needed to study the soil morphology and root dynamics (Baes, 1989). Considering the overall

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climatic conditions of the area, the young calcareous sandy deposits and a groundwater table between 65 and 225 cm from the soil surface, one would expect a well developed plant growth in the dune valley within 60 years. Preliminary prospection indicated that probably physical soil conditions, particularly packing of the sand grains, would be the primary limiting factor for plant growth. In order to check this hypothesis a methodology for quantitative and qualitative soil description, with special attention to root distribution, was developed. Emphasis was laid on physical soil factors such as thickness of the biologically active layer, bulk density, porosity, root penetration resistance, the significance of capillary rise for plant water availability and the importance of faunal activity for root distribution as possible causes for the vegetation stability. The organic matter content was investigated by measurement of horizon thickness and the physical fractionation of organic matter.

DESCRIPTION OF THE STUDY AREA

The research was carried out in the northern part of the 340 ha large State Nature Reserve "De Westhoek". The morphographic units are (Depuydt, 1967) (Fig. 1) (1) the fore-dune bordering the sea, (2) further landwards, till about 750 m, three large dune valleys bounded by (3) more or less stabilised parabolic dunes, which gently grade into (4) a large and active "wandering" dune which extends till about 1300 m landwards.

The investigated transect of 520 m length was situated within the large cen-

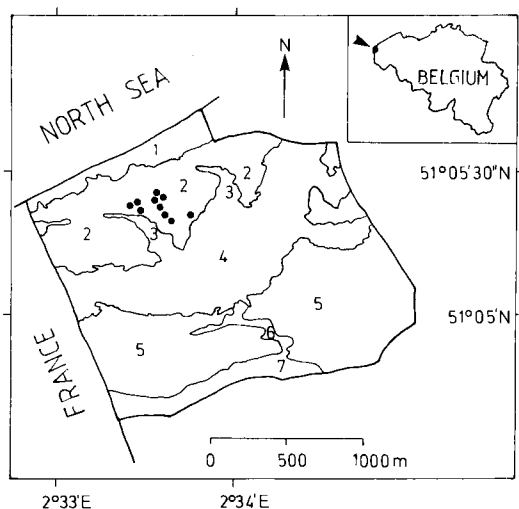


Fig. 1. The Nature Reserve "De Westhoek" in Belgium and its different morphographic units: 1=foredune; 2, 5=dune valleys; 3, 6=parabolic dune; 4=wandering dune; 7=southern dune wall; ● = profile pits.

tral dune valley. In the western part of the valley the soil surface was stabilised before World War II, the eastern part, bordering the wandering dune, was stabilised for only about 12 years (Fig. 1) (Ampe et al., 1991). The dune sands are well sorted, the mode of the particle size distribution varies between 175 to 195 μm . The CaCO_3 content varies between 6 and 10% (Depuydt, 1966).

Stabilised secondary microdune ridges (R) which are 40 to 130 cm higher than the valley bottom (DV) (Fig. 2) create a considerable microrelief. These ridges, oriented more or less parallel to the wandering dune, were formed in the past at the boundary between the bare toeslope of the wandering dune and the recently vegetated dune valley bottom. The sand was deflated from the

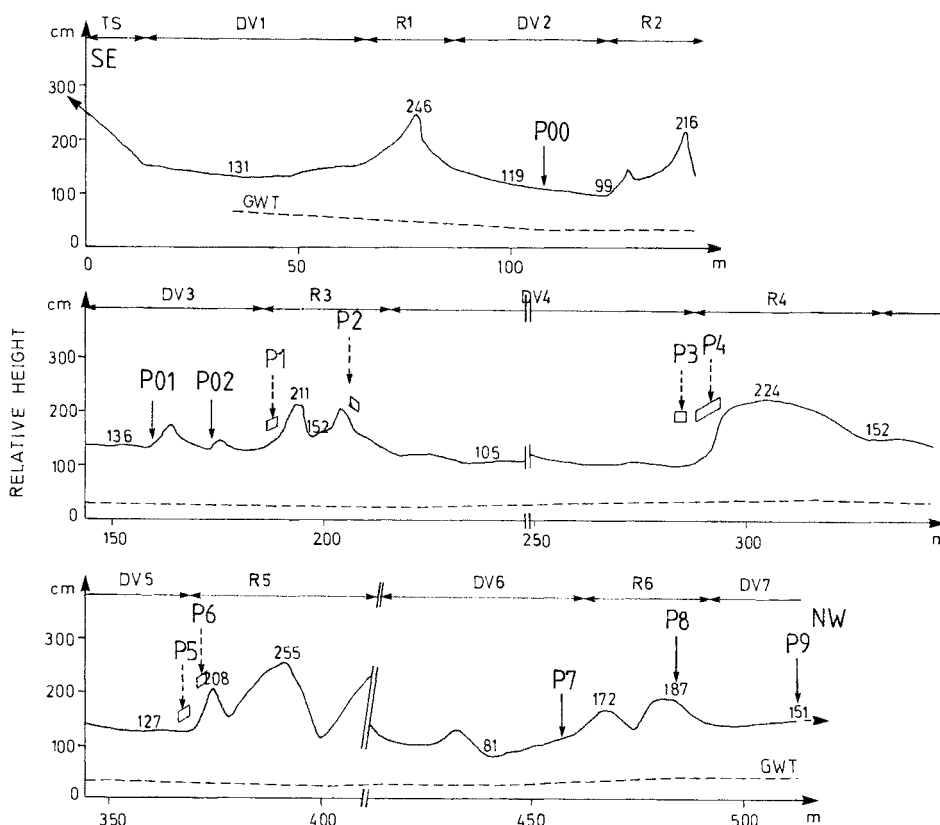


Fig. 2. SE-NW topographical transect along the chronosequence, based on 125 measurements, location of the profile pits, main geomorphic units and groundwater table. Key: *TS*=toeslope of wandering dune; *DV1,2 ...*=dune valley 1,2, ...; *TS* and *DV1* are subjected to active deflation; *R1,2 ...*=secondary microdune ridge 1,2, ...; *PO,1 ...*=profile pits and sampling sites *O,1, ...*; —=groundwater table (GWT) on 27-3-1991; ↓=position of profile pit and/or sampling site (dashed arrow=position of profile pit situated laterally of the transect); 131 ...=relative height (cm); vertical exaggeration = $\times 12$; ||=lateral but parallel displacement of traverse.

bare dune slope in short periods of northeastern winds, opposite to the dominant southwestern wind direction of the area (Ampe et al., 1991).

Most of the central dune valley is covered with a mixture of shrubs of *Salix repens*, *Hippophaë rhamnoides*, and *Ligustrum vulgare* which are between 1 and 2 m high. Exceptions are (1) DV2 (Fig. 2), stabilised for about 12 years (POO), with open *Salix repens* shrubs only 2–15 cm high; (2) P3, the wettest part of the chronosequence where *Hippophaë rhamnoides* shrubs attained an exceptional height of 3 m; (3) the highest part of P4, about 40 years old, with scattered *Populus candicans*, about 4 m high. P8 and P9 grazed till World War II, formerly a mosaic of mesophyll grassland, is at present nearly completely covered with dense patches of *Ligustrum* with a maximum height of 2 m.

The nearest meteorological station is Koksijde, (2°39'E, 50°5'N, +9 m), situated 2400 m from the beach and 4500 m from the study area. Annual rainfall (1957–1976) is 673 mm, annual potential evapotranspiration 485 mm and mean annual temperature 9.7°C (Lebbe, 1978). Moisture deficits occur from May till August. The study period coincided with the two particularly dry summers of 1989 and 1990, in which the months May to September 1989 and 1990 received about 71% and 56% of the average rainfall of that period.

Due to an excess of precipitation over evapotranspiration from September till March a fresh water reserve is present under the dune area (Lebbe, 1978). Groundwater fluctuations occur on daily, seasonal and annual basis. Lebbe and De Breuck (1980) measured differences ranging from 40 to 80 cm between lowest (September to October) and highest (February to March) water levels; yearly fluctuations of 60 to 90 cm were noted between more humid and more dry years.

METHODS

The relief of the 520 m long traverse (Fig. 2) was recorded based on 125 relative height measurements with a Kern levelling instrument. The successive dune valleys (DV) and secondary ridges (R) have been numbered from young to old, DV1 is still active, DV6 and DV7 were stabilised before World War II. The age of the stabilisation was estimated by D'Hondt (1981) based on airphoto interpretation for the period 1944–1977, complemented by De Raeve for the period 1977–1988 and verified by Ampe (1991).

Along the transect — which forms a chronosequence, since age of stabilisation decreases towards the wandering dune — ten pits, each representing a topo-hydrosequence on the secondary ridges, were dug (Fig. 2). The pits were 2.5 to 6 m long, 1 m wide and 90 to 190 cm deep. At representative sites in the pits, horizontal sections of 80×80 cm² were closely studied for each horizon. The profiles were described according to the ITC-Ghent Guidelines (Adiwiganda and Langohr, 1989) which allow to record relevant ecological

information on soil studies. The following horizon symbols were used: O1=not to slightly decomposed organic matter; O2=decomposed organic matter; A1=surface humus rich mineral horizon; Bbi=bioturbated B horizon; C=stratified parent material; d=dense; g=rusty mottling; G=presence of the permanent groundwater and b=buried horizons. The symbols of the sampling sites were recorded as follows: P00, P1, P2,... topo-hydrosequence pits; L=Low; M=Middle; H=High; Hip=*Hippophaë*; Sal=*Salix*. Detailed sketches of the vertical walls and horizontal sections were drawn with special attention to the amount, distribution, diameter, condition (live or dead) and species of the roots.

Along the chronosequence, at 17 sites, three samples of the surface horizons till about 10 cm deep were taken with a metal open box of $20 \times 20 \text{ cm}^2$. In the laboratory the samples were air dried and sieved into the following fractions: $> 2 \text{ mm}$, $2 \text{ mm} - 500 \mu\text{m}$, $500-250 \mu\text{m}$, $< 250 \mu\text{m}$. In the fraction larger than 2 mm, mosses, leaves, roots, twigs and undifferentiated organic matter were distinguished.

A first estimation of the thickness of the loose biologically active layers was made with a penetration rod, which has a cone-shaped surface area of 1 cm^2 and in a period when the soils were at field capacity (end April 1991). The rod was pushed with closed hand to determine the upper limit of the consolidated sands. Below this depth it was impossible to push the rod any further.

Undisturbed samples of the soil matrix were taken with an ELEY volumeter (30 cm^3) and analysed for bulk density (BD) and moisture content. The samples were taken under different weather conditions and at various distances from the groundwater table resulting in a high variability in water content. Yet as the samples contain nearly no silt nor clay the bulk density results are considered to be comparable. % Porosity (%POR) was calculated according to the formula: $\% \text{POR} = (1 - \text{BD}/\text{PD}) \times 100$. For particle density (PD) the value of 2.65 g cm^{-3} was assumed. Some samples rich in organic matter have a lower PD so that the %POR was overestimated here.

The height of the capillary rise was determined by taking undisturbed samples at 10 cm intervals from the groundwater table at the end of a long dry period.

Statistical techniques such as *F*-ratio test, Spearman rank's correlation coefficient, Mann-Whitney *U* test were applied as described by Hammond and McCullagh (1980).

RESULTS AND DISCUSSION

Soil morphology along the chronosequence

The studied profiles showed the following horizon sequence and characteristics (Fig. 3) :

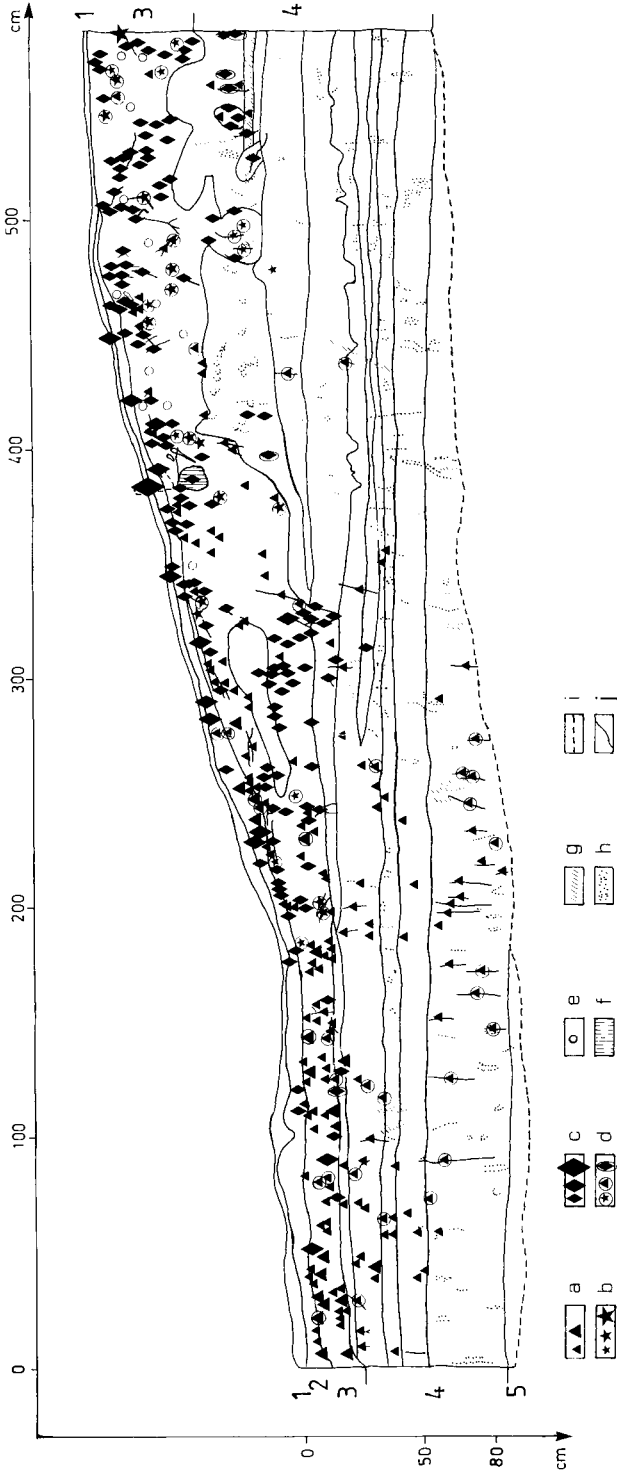


Fig. 3. Topo-hydrosequence P4 under *Salix* (depression), *Ligustrum* (midslope) and *Populus* (ridge). Orientation ESE-WNW. Key: 1 = O1 + O2; 2 = A1bi; 3 = Bbi; 4 = Cd; 5 = CGd; a = live roots of *Salix*; b = live roots of *Hippophaë*; c = live roots of *Ligustrum*; d = dead roots of *Hippophaë*; *Salix*; *Ligustrum*; e = dead unidentified root; f = mesh of roots; g = humified material; h = redoximorphic mottles; i = lower limit of the profile pit; j = dead or live root, parallel to vertical profile wall. The roots in the O and A1 horizon have not been recorded.

(1) Litter layer (O1 and O2) locally absent on the microdune ridges and up to 10 cm thick in the depressions, very few to abundant roots;

(2) A1bi, humus rich, locally absent on the microdune ridges and up to 14 cm thick in the depressions, abundant to very abundant roots;

(3) Bbi, loose biologically active layer, ranging from 4 cm thickness in the depressions to 71 cm in pockets and tongues up to 30 cm in diameter on the slopes and on top of the ridges, abundant to very abundant roots;

(4) Cd, relatively dense, not reduced sands, often horizontally stratified, locally with relic oximorphic iron linings along the pores, thickness ranging from 41 to 155 cm, few to moderately abundant roots;

(5) CGd, relatively dense, reduced sands, the depth of the upper limit depends on the topographical position, 65 cm in the depressions and up to 185 cm on the ridges, very few roots.

Data on the thickness of the loose material (O1 + O2 + A1bi + Bbi) determined with a penetration rod and on organic matter fractionation of the surface horizons are shown in Table 1. The transition between the loose surface horizons and the dense Cd horizons occurs within a distance of a few centimeters.

As long as young (P1L, P2L) and old (P9) sites are compared in similar landscape positions, a similar depth of loose horizons is recorded. On the ridges, such as P12H, P4H and P6H, the dense horizons started at a depth of 56, 46 and 41 cm respectively (Fig. 4). In the depressions these horizons started between 16 and 35 cm depth (Fig. 5). Consequently, the depth at which the dense horizons start seems to be independent of the soil age.

Litter and humus content vary more along the topo-hydrosequences than along the chronosequence (Table 1). Litter content is small on the ridges (P12H, P4H, P56 and P6H). The highest values were obtained in P3Sal, the lowest part of the transect. Also the humus content correlated with the topography with highest values in the lows (P3Hip, P3Sal, P4L and P5L) and the grazed systems (P7L, P7H and P9). The lowest humus contents were recorded on the ridges (P12H, P4H and P6H). The age factor plays a minor role as it only diversifies the samples of the very young systems (P00 — less than 12 years old) for the amount of litter.

The 17 sites where organic matter fractionation was done, were grouped into 5 systems based on the age of soil stabilisation. The *F*-ratio test was applied (Table 2) to check whether variability amongst the samples of the sites for each system is less important than the variability amongst the different sites for each system. For the younger sites (P0, P1-2) a low variability is shown amongst the samples per site and a high variability from one site to another; for the older systems (P7-9) where the *F*-ratio test is not significant, the variability amongst the samples per site is high; hence, it is not possible to maintain that samples come from different sites. Since age of stabilisation for the sites of one system is more or less equal, it is possible to explain the

TABLE 1

Upper limit of the consolidated sands measured with a penetration rod, thickness of the A1bi + Bbi measured on the vertical sections of the profiles, relative height of the soil surface above the reference level of the study site, air-dry weight of litter and humus

Site ^a	Penetration rod		Vertical sections	Relative height of soil surface (cm)	Air-dry weight (g)			
	Upper limit dense subs. depth ^b (cm)	hor. S.D. ^c			Litter ^d	S.D. ^c	Humus ^d	S.D. ^c
P00	23	3.30	20	108	32.4	13.07	83.3	12.74
P01	16	4.80	—	135	118.8	19.01	125.9	6.06
P02	22	3.61	—	137	93.6	9.00	100.0	21.00
P1L	24	6.71	25	169	76.4	19.60	85.6	26.27
P12H	56	13.91	65, 71 in pockets	206	77.4	11.90	39.4	5.72
P2L	22	4.43	29	124	127.0	7.19	132.5	25.12
P3Hip	18	3.34	23	95	138.1	35.61	172.5	27.69
P3Sal	21	4.84	23	94	178.5	9.22	184.8	15.88
P4L	23	3.00	25	103	172.4	21.78	224.3	20.25
P4M	35	7.30	46	129	110.9	39.80	144.2	0.85
P4H	46	10.58	44, 66 in pockets	185	102.0	18.70	91.1	7.20
P5L	35	3.15	20	152	105.9	24.84	191.1	42.14
P56	37	9.39	40	185	82.1	23.10	72.9	16.69
P6H	41	14.88	25, 55 in pockets	223	92.2	19.09	77.5	3.72
P7L	27	1.93	20	117	139.3	22.49	216.8	57.48
P7H	27	7.40	20	164	114.4	28.64	190.9	43.73
P9	27	7.21	22, 28 in pockets	157	90.1	14.05	231.9	25.02

^aL: Low, M: Middle, H: High, Hip: Hippophae, Sal: Salix.

^bValues are an average over 15 readings.

^cS.D. = standard deviation.

^dValues are an average over 3 readings, each over a 20 × 20 cm² surface and for the whole thickness of horizons O + A1.

variability amongst the sites of the younger sites in terms of relative height. The older systems show a much larger variability amongst the samples themselves which have to be explained by other events occurring in the past, such as grazing (D'Hondt, 1981), subsequent vegetation changes when grazing was abandoned and bioturbation.

Overall it can be concluded that the soil morphology as well as the soil organic matter remain remarkably stable throughout the transect — except for a certain variability according to microtopography and distance to the groundwater table. The most striking characteristic along the chronosequence is the limited and nearly constant depth of the biologically active horizon, a feature neglected in previous edaphic studies of dune ecosystems.

Physical soil properties

Physical properties and their statistical characteristics are given in Table 3. The mean bulk density was lowest in the A1bi horizons, followed by the



Fig. 4. Vertical section through P4H; top of ridge; ± 40 years old; under mixed shrub of *Populus* and *Ligustrum* about 2 m tall; with biologically active (Bbi) horizon, undulating lower boundary, depth between 25 and 45 cm. The oblique 5 cm wide gallery between 55 and 65 cm depth on the left half of the picture is a former faunal gallery filled up with *Hippophaë* seeds stored probably by mice and penetrated by numerous fine roots; at 80 cm and 1 m depth, buried surface horizons (bA1).

buried organic horizons (bA1) and the biologically active B horizons (Bbi). The standard deviation is highest in the A1bi horizons and is much lower in the compacted Cd horizons which show a similar degree of compaction

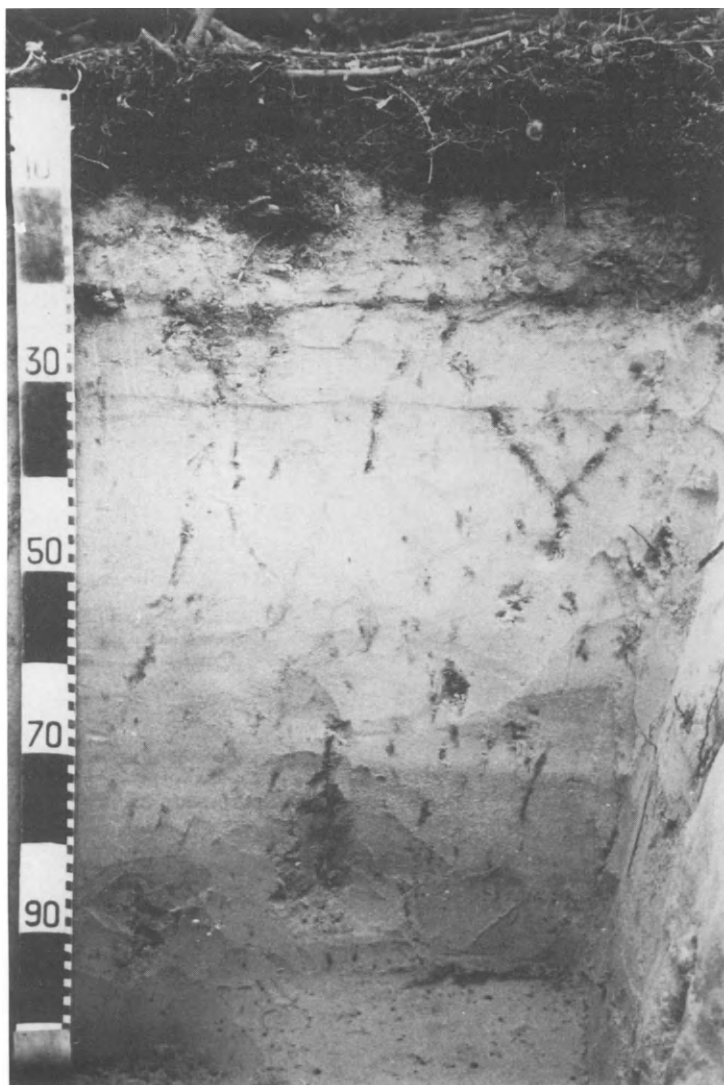


Fig. 5. Vertical section through P9; in valley bottom more than 60 years old; under dense *Ligustrum* shrub about 1.5 m high; the biologically active layer only 20 cm thick and smooth lower boundary; the deeper roots of *Ligustrum* grow vertically and predominantly in former root and stem galleries possibly of willow.

throughout the entire chronosequence.

Wiersum (1957) showed that in a rigid system a root is able to penetrate only those pores which have diameters exceeding that of the young root. Besides the diameter of the pores the rigidity and continuity of the pores are important (Aubertin and Kardos, 1965). As long as the root can displace soil

TABLE 2

Results of the significance level according to the *F*-ratio test

Sites within each system ^a	P0	P1L,P12H,P2L	P3Sal,P3Hip,P4L,P4M,P4H	P5L,P56,P2H	P7L,P7H,P9
Litter	0.005	0.05	—	—	—
Humus	0.1	0.05	0.005	0.01	—

^aAll systems, except P3Sal to P4H contain 3 sites, each site being sampled 3 times. P3Sal to P4H contains 5 sites, each site was sampled 3 times.

TABLE 3

Statistical characteristics of bulk density (BD) and porosity (%POR)

	BD(g cm ⁻³)	%POR
<i>A1bi horizons (n=44)</i>		
Mean	1.35	49
S.D.	0.14	5.1
<i>Bbi horizons (n=121)</i>		
Mean	1.50	43
S.D.	0.08	3.0
<i>Cd horizons (n=436)</i>		
Mean	1.56	41
S.D.	0.04	1.5
<i>bA1 horizons (n=75)</i>		
Mean	1.45	45
S.D.	0.08	2.8

n = number of samples.
S.D. = standard deviation.

particles it will be able to grow and to force a passage even when the original pores are smaller than the root diameter. Aubertin and Kardos (1965) found that maize roots did not grow into rigid pore systems which had diameters of about 138 μm . If we assume a loose stapling of the particles with a BD of 1.38 g cm^{-3} , a porosity of 48% and a particle diameter of 188 μm — the modal value for the dune sands of the study area (Depuydt, 1966) — then the average pore diameter is about 78 μm . This is markedly smaller than the diameter of rigid pores as proposed by Aubertin and Kardos (1965). Since the above calculation is based on the assumption of a loose particle arrangement, the pores will not be rigid and roots will be able to move the soil particles during their growth. As the compact sands have a BD of about 1.56 g cm^{-3} the rigidity of the pores will be much more important and root growth will be strongly inhibited.

The average bulk density for all the Bbi horizons (*n*=91) and the adjacent underlying Cd horizon (*n*=77) were 1.50 and 1.56 g cm^{-3} respectively. The

Mann–Whitney *U* test showed that the bulk density of the Bbi samples were lower than those of the Cd horizons at a significance level of 0.001. Considering that in most cases the transition between the Bbi and Cd horizons occurs within a distance of less than a few centimeters (the average sampling depths were 20 and 33 cm respectively), the overburden pressure is eliminated as a possible explanation for the higher density in the Cd horizon.

Amongst numerous processes (Pye, 1983; Sands et al., 1979) causing compaction once aeolian sands are deposited, only wetting and drying could play a role in the study area. Poelman et al. (1989) found higher bulk densities in mottled horizons of cover sands with median 170 μm (1% clay, 5% silt) which was thought to be caused by periodic wetting and drying. The alternating cycle would loosen the silt bonds between the sand grains during the wetting and cause the collapse of the grain system during drying. The process is irreversible due to the absence of swelling particles in these materials. The above data show that the packing of the sands has critical values for root penetration which already occur in these soils from a few decimeters depth.

The mean %POR (Table 3) was 49%, 44% and 45% for the A1bi, Bbi and bA1 respectively. For the Cd layers the %POR amounted to 41%. In a similar sandy soil, De Haan and Van der Valk (1970) found that at a %POR of less than 42 to 43%, root growth did not occur. This is in agreement with our results.

At the end of the long dry summer of 1990 a series of samples were taken at the level of the groundwater table and at regular distances of 10 cm upwards. The results (Table 4) show that the capillary rise reaches a height of about 40 cm in the dense subsurface horizons (Cd) with a mean bulk density

TABLE 4

Average physical soil properties of 5 samples with groundwater table at 155 cm depth, profile pit P5

Depth from surface (cm)	BD (g cm ⁻³)	S.D.	%WV	S.D.	%WW	S.D.	%POR	S.D.
65	1.56	0.026	0.9	0.72	0.5	0.46	41.1	0.97
75	1.58	0.007	1.5	0.64	0.9	0.42	40.3	0.30
85	1.58	0.012	3.2	0.52	2.0	0.33	40.3	0.43
95	1.60	0.018	6.9	0.79	4.3	0.48	39.6	0.68
105	1.61	0.023	15.4	0.79	9.6	0.70	39.3	0.89
115	1.63	0.010	30.7	1.51	18.8	0.84	38.6	0.38
125	1.59	0.016	36.7	2.69	23.0	1.63	39.8	0.65
135	1.60	0.007	38.9	1.01	24.4	0.57	39.7	0.30
145	1.49 ^a	0.038	40.3	4.10	27.1	3.13	43.8	1.41
155	1.43 ^a	0.010	46.3	0.83	32.3	0.48	45.9	0.37

^aBuried organic horizons.

Abbreviations: BD, bulk density; %WV, % water by volume; % WW, % water by weight; %POR, % porosity; S.D., standard deviation.

of 1.59 g cm^{-3} . This figure is very close to the 42 cm found by laboratory testing (Michiels et al., 1989) on dune soils with comparable texture and a similar sand fraction just south of the nature reserve. From these data it is evident that a drop of the groundwater table of more than 40 cm below the lower limit of the biologically active layer will have an important impact on the water supply for plant growth. This explains the drought stress and the stunted shrub growth only 1.5–2 m high after 60 years of stabilisation, even in most of the lower landscape positions of the transect. Only P3, with a permanent groundwater table at a depth of 65 cm and a lower limit of the Bbi at 18 to 21 cm seems to cope with these drought problems even in the dry summers of 1989 and 1990.

The general position of the groundwater table at more than 40 cm below the lower limit of the shallow Bbi throughout the chronosequence explains that plant growth is limited, although groundwater is present within relative short distance from the soil surface.

Rooting characteristics

The study of the vertical sections (Fig. 3) clearly showed that most roots are concentrated in the A1bi and Bbi horizons. Horizontal roots were dominant in the upper 20 cm in the depressions and up to 65 cm in the ridges. This depth may reach 71 cm (Table 1) (Fig. 4.) in pocket or tongue-like features filled with loose sand and many roots. These were interpreted as former faunal (rabbit) galleries as all intermediate phases from open to completely but loosely filled galleries could be observed. At a greater depth in the stratified sands of the Cd horizons, horizontal roots diminish drastically (Fig. 6).

In the Cd horizon, most roots are growing vertically and only the horizontal sections permitted to count and record the roots.

Still a rather high total (live, live in dead, dead) number of roots are observed within the dense Cd horizons of P12H, P2L, P3Hip, P3Sal, P4L, P5H, P7 and P9. The horizontal sections showed that many of these living roots were growing within a former root or stem gallery (Fig. 7), recognizable by the presence of weak to strongly decomposed organic matter. Locally the original plant species could still be recognised by the presence of the undecomposed bast of the root or stem. Table 5 summarises these data for all the horizontal sections. A selection from the raw data (Ampe, 1991) showed for example that at P3Hip, P3Sal, P4L, P5H and P9 a total of 31 live roots occurred at 65 cm, 52 roots at 71 cm, 171 roots at 67 cm, 115 roots at 88 cm and 98 roots at 66 cm in the respective sites, whereby between 77 to 100% of the roots were growing within an older one; at these depths the bulk density varied between 1.58 and 1.61 g cm^{-3} . Species observed growing through the old root channels are *Salix* and *Calamagrostis*, to a lesser extent *Ligustrum* and occasionally *Hippophaë*, *Rubus* and *Epilobium*. The rooting pattern illus-

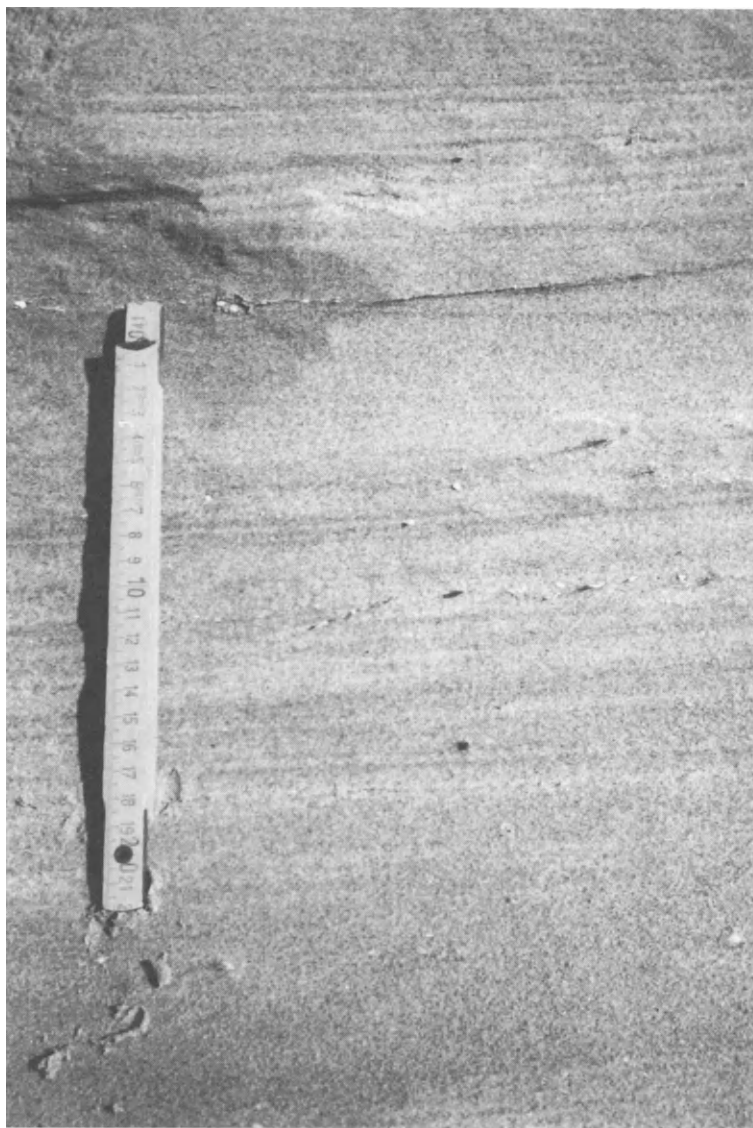


Fig. 6. Cd horizon with closely packed stratified sands and no plant roots or stems. The penetration resistance of this material is too high for root growth (scale is 20 cm long).

trates the difficulty of rooting development within the Cd horizon where faunal galleries and pre-existing root or stem galleries correspond to the major pathways through which roots can penetrate deeper into the soil.

The presence of live roots in dead ones can be explained in terms of the dune building history: dune valleys are blown out by dominant southwestern winds till the capillary zone of the groundwater table is reached. At this point



Fig. 7. Same horizon as Fig. 6 but in an area where *Ammophila* was growing during the dune building. When the *Ammophila* died, its stem galleries are the only spots where roots of newly colonizing plants penetrate deeper into the soil and may reach the vicinity of the groundwater table (scale is 20 cm long).

fixation of the dune valley takes place mainly by *Salix repens* and *Carex* species. On the edges of the dune valley opposite to the parabolic dune the secondary microridge is characterised by the presence of *Ammophila* and *Salix* which are able to grow in pace with the fast accumulating sand. To a lesser

extent the same process takes place in the dune valley itself but here the “dune builder” is *Salix*. In a relatively short period these sands become more dense, most probably due to alternating wetting and drying cycles (Poelman et al., 1989), to an extent where the mechanical impedance for most plant roots is exceeded. In these sands, this seems to correspond to a bulk density exceeding 1.53 g cm^{-3} (Table 5), which is in agreement with the $1.51\text{--}1.54 \text{ g cm}^{-3}$ of De Haan and Van der Valk (1970). By then penetration will only be possible along the more or less vertical channels left by the stems and roots of the former dune builders. The locally high amount of such roots at substantial depths explains the survival of *Salix* even during the dry summers of 1989 and 1990. Indeed mainly *Salix* and grasses and to a much lesser extent *Ligustrum*, *Hippophaë* and *Rubus* grew in the existing channels. *Ligustrum* and especially *Hippophaë* had much more survival problems. This can be related to the root morphology of these two species. Close to the soil surface *Ligustrum* rhizomes, seldom more than 1 cm in diameter, grow horizontally to subhorizontally. The rhizome has a fibrous root system which forms a very dense mesh especially in bioturbated pockets and in the loose A1bi and Bbi horizons; hence, it is able to colonize the loose soil volume at a maximum. Compared to *Salix* and *Ligustrum*, *Hippophaë* has much less horizontal roots and possesses a very robust secondary vertical rooting system with relatively little branching. Therefore the soil volume which is exploited by *Hippophaë* is limited. It was also *Hippophaë* which had considerable survival problems during the dry summers of 1989 and 1990. It can be concluded that the growth of most live roots of former galleries points to the importance of the dune building history in the understanding of the subsequent plant growth.

CONCLUSIONS

Along the chronosequence, soils showed in general a rather similar sequence of O1, O2, A1bi, Bbi, Cd and CGd horizons. The thickness of the O1, O2, A1bi and Bbi horizons and the litter and humus content varied with the topographic position. According to the penetration rod measurements and the vertical profile sections, the loose surface horizons ranged in thickness from 16 to 35 cm in the depressions and from 22 to 65 cm in the ridges. Gradual dune growth with simultaneous plant growth and faunal (rabbit) activity caused deeper loose surface horizons in the ridges.

There is an abrupt transition between the Bbi and Cd horizons: the mean bulk density of the Bbi horizon was significantly lower ($\alpha=0.001$) than the underlying Cd horizon. It was observed that most roots were concentrated in the A1bi + Bbi horizons. The horizontal sections revealed that live roots may grow in the Cd horizons, but preferentially in vertical galleries of dead and decayed roots.

The dune building history appears to be of primary importance to the

amount and distribution of root growth at a depth of more than about 25 cm. Only a few plants such as *Ammophila* and to a lesser extent *Salix* are able to grow upwards together with the fast accumulating sand. At a later development stage *Salix* and grasses and occasionally *Ligustrum*, *Hippophaë* and *Rubus* explore the pre-existing root and stem galleries, hence, increasing their supply of water and nutrients as they reach a greater depth. As such these plant individuals have better survival chances during the dry spells.

The capillary rise in these sandy soils with a modal particle diameter of 188 μm and an average bulk density of 1.59 g cm^{-3} was about 40 cm above the groundwater table. As rooting was limited to mainly the A1bi and Bbi horizons, plant growth was subjected to water stress, especially in dry summer periods when the water table dropped so deep that the capillary rise did not reach the lower limit of the Bbi horizon anymore.

Vertical and horizontal sections are both mandatory for a three-dimensional observation of the root distribution. These reveal the importance of faunal activity as burrowing animals may counteract the process of densification of the sandy soil material. They also reveal that the knowledge of the dune building history — with or without dune building vegetation — is crucial in the later colonization ability of the stabilised soil.

The study allowed to come to a much better understanding of the studied ecosystem dynamics. It appears that the limited soil volume for root penetration is one, if not the primary limiting edaphic factor which permits to explain the very stable plant physiognomy with a shrub vegetation height of not more than 2 m, even after 60 years of stabilisation and growing on calcareous sands, with a soft groundwater at 65–225 cm depth.

The uniform plant physiognomy over microdune ridges with a permanent groundwater table up to 225 cm deep and lower landscape positions with a permanent groundwater table up to 65 cm seems to be largely related to the thickness of the biological active A1.bi+B.bi horizons. The drought problems of the higher landscape positions are partly compensated by a biologically active layer which as a result of faunal activity is at least double in volume as compared to the nearby lower landscape positions. Factors, such as diseases, occurrence of plant-parasitic nematodes and mycorrhizae, certainly play a role in the vitality of the vegetation. Yet these factors can hardly explain the remarkably uniform vegetation physiognomy in both the chronosequence and the topo-hydrosequences of the dune valley of the Westhoek Nature Reserve.

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Transformation of the soil structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract

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ABSTRACT

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Pontoscolex corethrurus is a geophagous tropical earthworm whose population in a Mexican pasture can ingest annually 400 Mg of dry soil ha⁻¹. A comparative study of the soil before ingestion, the gut content and casts of *P. corethrurus* using transmission or scanning electron microscopy (TEM and SEM) showed that the soil structure was completely destroyed. In the control soil the organo-mineral material was compact, while the soil in the anterior gut was dispersed in a semi-liquid medium with a great amount of free polysaccharides (intestinal mucus) and swollen clays by the increase of water content. At the end of the gut and in the casts the soil had retaken a similar structure than before its ingestion. Although in the gut a great reorganization of the soil occurred, as formation of new aggregates like microbial colonies surrounded by polysaccharides and clays, or activation of some dormant microorganisms. These observations supported with other physico-chemical (pH, water content) and microbial activity measurements showed how *P. corethrurus* had a dramatic impact on soil macro- and microstructure. This destructuration and restructuration of the soil made that organic elements that were protected were recycled and others, on the contrary, were protected by the formation of new microaggregates.

INTRODUCTION

Earthworms are known to be the most important soil fauna biomass in many humid soils of temperate and tropical regions (Lee, 1985). In addition their burrowing and great soil ingestion have been studied in relation to soil porosity, compaction, water fluxes, macrostructure. Lee and Foster's (1991) re-

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view on soil fauna and soil structure emphasize again the role of earthworms. Some works dealt with direct observations of the casts structure (Shipitalo and Protz, 1988, 1989; Marinissen and Dexter, 1990; Blanchart et al., 1990, 1993), strength of the aggregates (McKenzie and Dexter, 1987), nutrient richness and biological activity (Scheu, 1987). However, few studies have been carried out on the transformation of the soil during its passage in the gut of geophagous earthworms. These worms ingest great amounts of soil (Lavelle, 1978; Lavelle et al., 1989) and the effect of their population on the macro- and microstructure of the soil is significant.

The aim of this work was to compare the control soil, the gut content (anterior, middle and posterior) and the casts of *Pontoscolex corethrurus* by ultrastructural observations (TEM, SEM). In this way it was observed how organic matter and microbial cells and soil particles were affected and reorganized by their passage through the gut.

MATERIALS AND METHODS

P. corethrurus is a geophagous endogeic species with a pantropical distribution; the individuals were collected in a pasture from east Mexico in the state of Veracruz, where the population per ha was 1.5 million individuals and its annual soil ingestion was 400 Mg of dry soil (Barois and Lavelle, 1986). The soil was a Vertisol having a clay content of 20–45% and a field capacity of 38%. The C content of bulked soil from the upper 10 cm was 4.7% and the C-to-N ratio 13.

Clitellate worms were cultured under laboratory conditions at a temperature of 26°C, in the 0–10 cm soil layer from the pasture; the latter sieved at 2 mm, moistened at field capacity. The worms were killed in boiling water (2 s) just prior to their fixation in glutaraldehyde. After fixation the guts were dissected and divided into anterior, middle and posterior thirds. The control soil was also from the 0–10 cm layer, set with the same conditions than the soil of the laboratory cultures, but without earthworms and fixated after 24 hours of incubation at 26°C. The casts, easily recognized from the surface of the sieved soil, were collected and had an average age of 12 and 36 hours.

Scanning electron microscopy (SEM) was carried out on the control soil and casts, on samples (3 mm³) which were dried in a dessicator prior to gold coating.

Transmission electron microscopy (TEM) of five samples, the control soil, the three sections of the gut and casts of *P. corethrurus* was carried out. The TEM technique of Villemin and Toutain (1987) has the advantage of not disturbing the samples. The samples of 3 mm³ were fixed in glutaraldehyde, dehydrated and embedded in resin. Samples were sectioned with an ultramicrotome, placed on a lattice and stained with uranyl acetate and lead citrate. Polysaccharides were contrasted by the silver proteinate sequence of Thiery

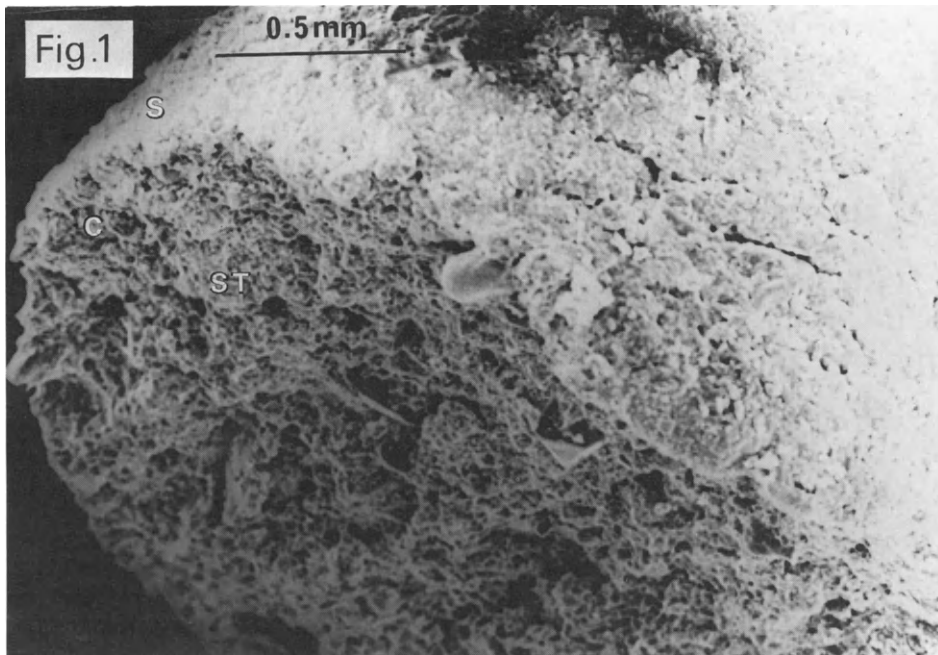


Fig. 1. An overview of a sectioned cast (SEM), presence of a smooth external surface (cortex). *B*=bacteria, *BP*=dark pigment, *C*=cortex, *Ca*=canals, *Cl*=clays, *Cl_s*=swollen clays, *HH*=humified hyphae, *Lz*=area of lysis, *M*=mineral particle, *OM*=organic matter, *OMM*=organo-mineral matrix, *OMH*=humified organic matter, *P*=polysaccharides, *Pm*=polysaccharides, intestinal mucus, *S*=external surface, *ST*=transversal section.

(1967). More than three sections and 20–60 fields were observed per sample, giving more than 220 observations.

RESULTS

SEM

The differences observed between the casts of *P. corethrurus* and the control soil were at the external surface where it is smooth (Fig. 1) for the casts and irregular for the control soil. In the casts there was also a difference of contrast among the external surface and the internal material which has the same colouring and appearance as the control soil surface.

TEM

Control soil

The control soil showed a very tight organo-mineral mixing although on the whole the structure was not compact. There were coarse particles of min-

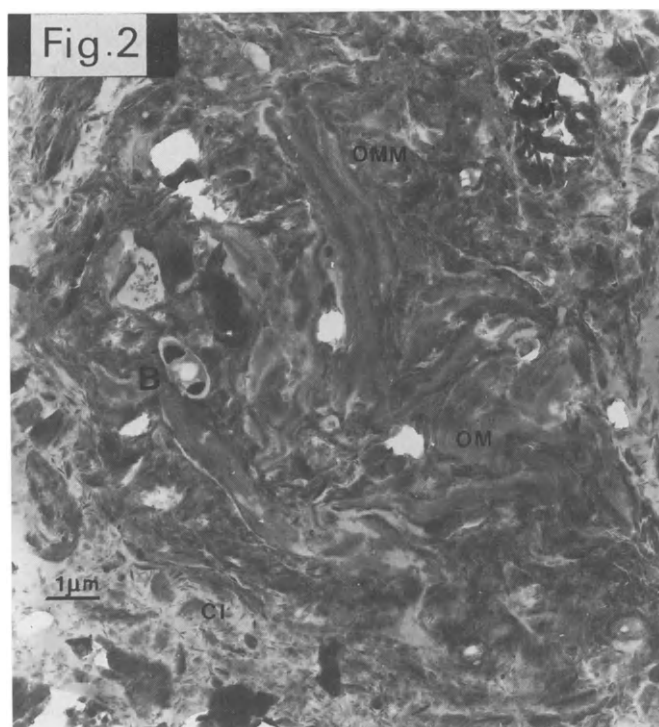


Fig. 2. Soil before ingestion, an over view (TEM): presence of humified organic matter with adsorbed clays, active free bacteria. For legend see Fig. 1.

eral, clays, plant debris (cells, polyphenols, vessels) and microorganisms (Fig. 2). Some active bacteria had excrescence of fibrillar polysaccharides which reflected microbial activity (Barois et al., in prep.) others formed aggregates by the adsorption of clays on to their polysaccharide sheath produced for their protection.

Gut content

The anterior section of the gut showed a less compacted environment, than the control soil (Fig. 3). The organic and mineral materials were dispersed in an organo-mineral matrix. This matrix was constituted of a mixture of water and intestinal mucus secreted by the worm, and the clays were swollen by the water increase. The water and mucus contents of the soil in this section were 150% and 16% respectively, while in the control soil they were only 35% and 0.12% respectively (Barois and Lavelle, 1986). The mucus can be seen as black dots developed by the Thiery staining in Fig. 3.

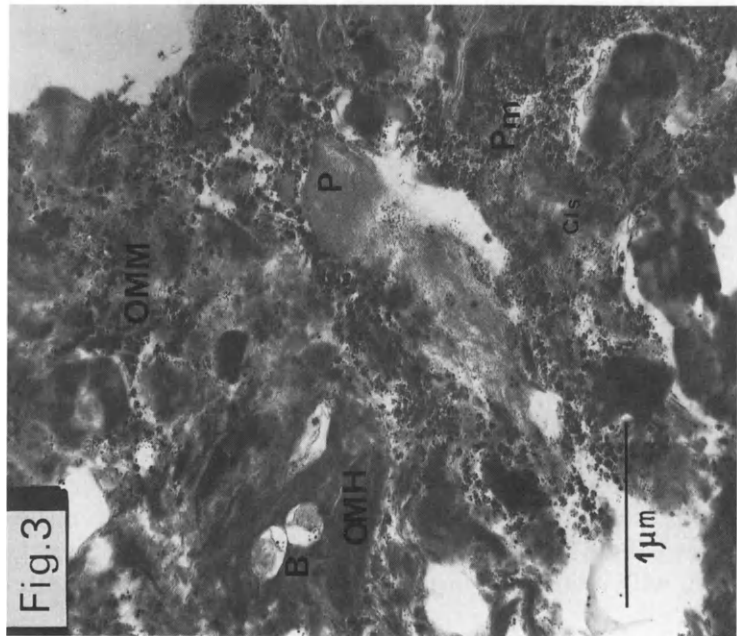


Fig. 3. Anterior gut content (TEM): semi-liquid media with presence of intestinal mucus rich in polysaccharides (black dots, Thiersy stain) and swollen clays. For legend see Fig. 1.

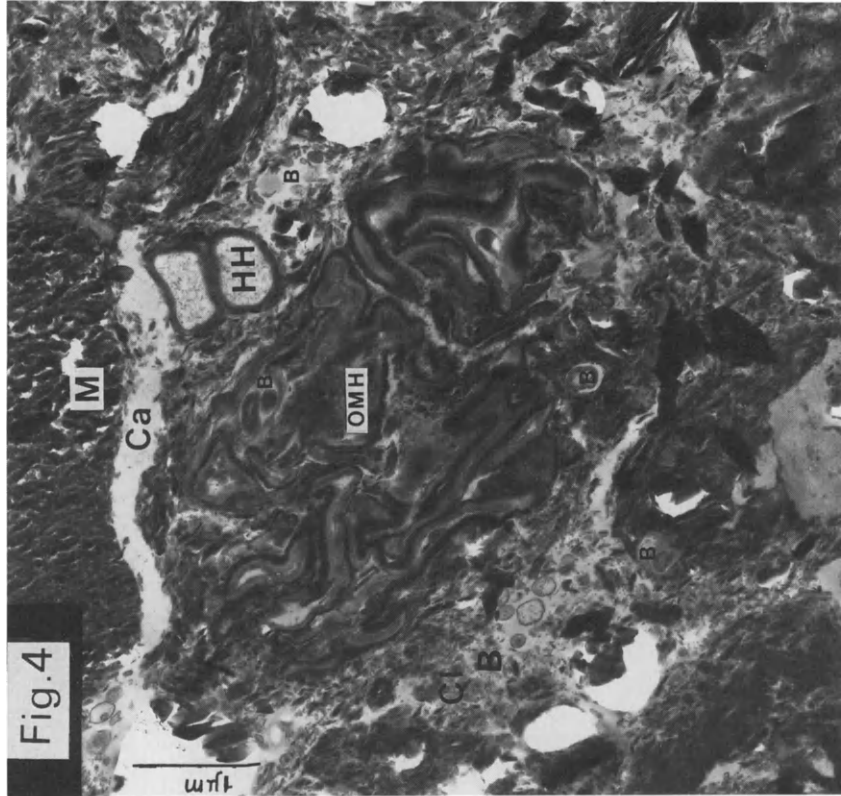


Fig. 4. Middle gut content (TEM): restructured media with a canal, rich in humified organic matter (fungi hyphae) , some bacteria are active. For legend see Fig. 1.

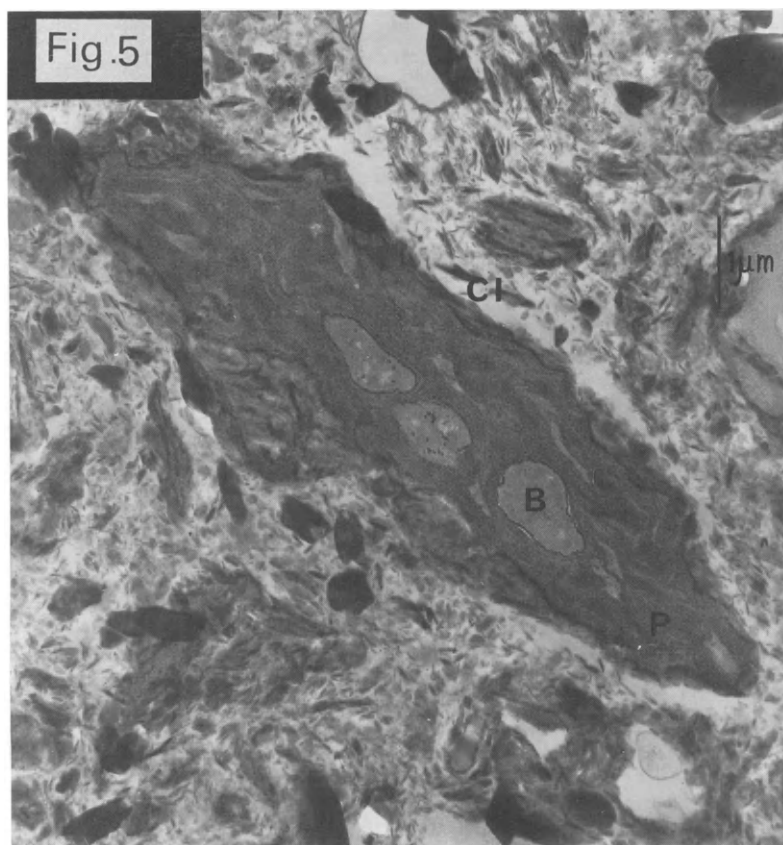


Fig. 5. Posterior gut content (TEM): bacterial microaggregate, Gram-bacteria with lobulated cell wall forming a polysaccharidic sheath surrounded by adsorbed clays (Thiery stain). For legend see Fig. 1.

In the middle and posterior gut content the matrix was dense and the organic and mineral particles were packed together (Fig. 4). Some bacterial colonies developed with a thick sheath of polysaccharides around them on which the clays were adsorbed thus forming microaggregates. Some bacteria of these colonies had lobulated cell walls (Fig. 5) which is characteristics of Gram- bacteria (Kilbertus, 1980) that were already known to form aggregates (Hattori, 1988). There were also free bacteria, spores of bacteria, and actinomycetes and protozoa cysts. The fungi hyphae were dead and very melanized and some drops of polyphenols (dark pigments) were present, resulting from decomposition and humification processes (Fig. 4). The clays were again as in the control with a lamellar form and adsorbed on organic matter (Fig. 5).

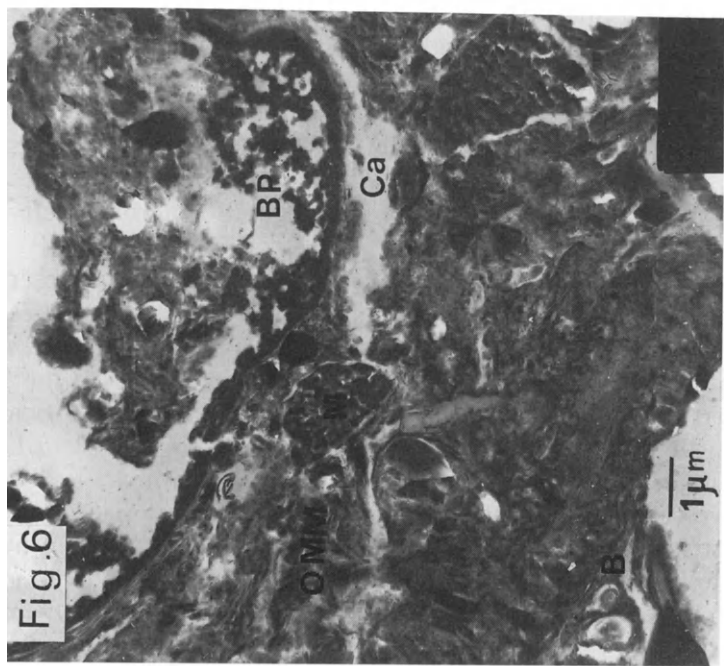


Fig. 6. Cast, an over view (TEM): structure similar to that of the control soil, dark pigment highly decomposed, rounded mineral particle. For legend see Fig. 1.

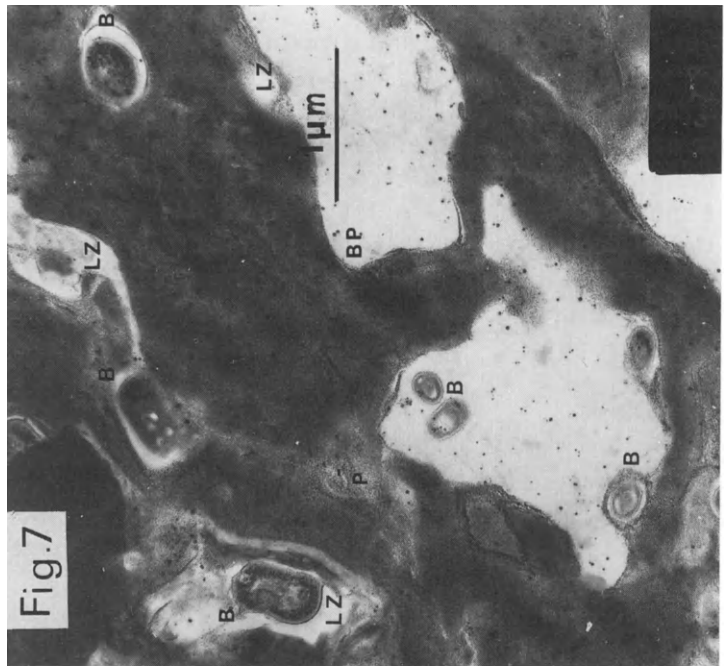


Fig. 7. Cast (TEM): active free bacteria in dark pigment with area of lysis and rich in polysaccharides (Thiery stain). For legend see Fig. 1.

Casts

In the casts the matrix had a more dense and structured aspect than in the gut content and there were canals and pores (Fig. 6). The microflora seemed to be very active because there were a few spores and many free active bacteria in activity decomposing organic matter and showing areas of lysis around them (Fig. 7). On the whole the observed bacteria of this sample were not surrounded by clays and were of smaller size than the bacteria that formed microaggregates. In the oldest cast (36 h) the microbial activity was less, there were spores, dead bacteria and others developed bacteria, particularly those having excrecences of fibrillar polysaccharides; fungi hyphae dead with a very melanized cell wall and other alive.

DISCUSSION AND CONCLUSION

In SEM the casts were observed like units or isolated aggregates differentiated from the bulk soil by their external surface. The latter had been defined by Blanchart et al. (1993) as the cast cortex being the result of the arrangement of clays and organic material.

In TEM observations some tendencies were perceived for the soil microflora and organic matter, and their dynamic in the gut content of *P. corethrus*. The microflora observed in the gut was mainly of soil origin, excepted for a bacteria population noticed coating the gut wall (Barois et al., in prep.). In the gut content there was less spores and hyphae than in control soil and in the casts. The active soil microflora in the gut content was apparently not active in the control soil and the casts and vice versa: in fact the spores and active bacteria observed in the gut were not the same as in the external environment. Along the gut transit there was an increase of the bacterial activity, with formation of bacterial colonies which in the posterior gut constituted microaggregates by clays adsorption around. Thus, it was confirmed by respirometry measurement showing that in the posterior gut the microbial activity was 6 times higher than in the control soil (Barois and Lavelle, 1986)

The earthworms select some mineral particles, because in the TEM observations minerals of rounded form and with a diameter of 2–6 μm were present in all the samples. Martin (1989) did the same observation on *P. corethrus* with the technique of physical fractionation. The comparison of the 5 samples showed that there were processes of biodegradation and humification taking place during the passage through the gut, but in the casts identifiable organic structures, like hyphae or vegetal vessels still remained.

In this study, nevertheless the most remarkable fact was the impact of the worm on the soil structure. Although the sieving at 2 mm of the control soil did not affect the soil microstructure, while it was completely destroyed in the gut. The destruction occurred first by the grinding of the soil in the gizzard and then, in the anterior gut by the high increase of water content and poly-

saccharides (intestinal mucus) which acted as "suspension dilution" agents. In the middle and posterior gut the content had less water and looked more compacted. In the casts, the soil structure was similar to that of the soil before its ingestion. Although during the gut transit old microaggregates were destroyed and new microaggregates were formed. The soil organic matter went through many transformations: biodecomposition, neoformation (humification) and releasing or protection by mineral particles (clays).

The restructuration of the soil after its passage through the gut of *P. corethrurus* constitutes a kind of "rejuvenation" or "regeneration" of the soil. Thus the geophagous earthworms by their great amounts of soil ingestion have a true impact on the structure. It has to be noticed that this process of destruction and restructuration takes place in a few hours (2–8). However, Shipitalo and Protz (1988) and Marinissen and Dexter (1990) measured that the fresh casts of *Lumbricus terrestris*, *L. rubellus* and *Aporrectodea caliginosa* had higher dispersion index than the dry casts and that there were possibilities of soil erosion during the cast were drying; but they confirmed the idea of soil structure renovation. Another aspect this work showed was the importance of the organic matter in the processes of aggregation. In the whole study organic matter was the pole of attraction of minerals and the center of any aggregations.

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Influence of fir root zone on soil structure in a 23 m forest transect: the fractal approach

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ABSTRACT

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This paper reports the results of a joint colloidal chemistry and fractal approach to the study of aggregate structures in the A_1 (0–10 cm) and $A_1(B)$ (20–30 cm) horizons of a sandy acid brown soil collected along a 23 m transect under three fir trees (eastern France, declined fir ecosystem).

The amounts of both organic carbon and $Al_{oxalate}$ clearly discriminate between the two horizons studied but no significant root zone differences were observed. However, the A_1 horizons collected in the fir root zone contained much more polysaccharides than the corresponding non-root zone horizons, and root zone soil polysaccharides had both a plant and a microbial origin.

The impact of a poorly-ordered Al hydrous oxides–organic matter balance (Al_{ox} –C significant negative correlation) on both physico-chemical and structural characteristics of soil millimetric aggregates was demonstrated.

The soil zero point of charge (ZPC) was mainly correlated both with positively charged poorly-ordered Al hydrous oxides and with negatively charged organic matter:

$$ZPC = 7.3Al_{ox}(\%) - 0.14C(\%) + 3.69 \quad (r=0.85; P<0.001)$$

More porous and water-stable aggregates as fractal domains over a larger range of spatial scales were observed in the A_1 organic horizon rather than in the deeper Al-rich $A_1(B)$ horizon.

Finally, the surface fractal dimension D_s (as determined by mercury porosimetry) was found to be a better discriminator than the porosity between the root zone and non-root zone of the soils.

INTRODUCTION

In his pioneering work on grass roots–soil structure interactions, Tri (1968) demonstrated that granulation occurred either through fragmentation of soil

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aggregates into smaller structural units or through aggregation of microaggregates, depending on whether the soil was originally compact or loose. He also showed that the average size of soil rhizosphere aggregates was correlated with the root density in the soil horizon studied.

More recently, Tisdall and Oades (1982) proposed a hierarchical model of soil structure formation in which inorganic and relatively persistent organic binding agents are important to the development of $<250\ \mu\text{m}$ microaggregates which are themselves aggregated in larger structural units ($>250\ \mu\text{m}$ microaggregates) by physical entanglements from grass and crop roots and mycorrhizal fungal hyphae.

This model was supported by the results from Thomas et al. (1986) who found that onion rhizospheric soil inoculated with mycorrhizal fungi had more aggregates and was more porous than uninoculated rhizospheric soil. These authors observed a positive correlation between root mass and aggregate weight, suggesting a direct control of rhizospheric aggregation by roots.

Similarly, Miller and Jastrow (1990) demonstrated that root lengths of prairie grasses and of perennial species of Compositae were positively correlated with the geometric mean diameter of aggregates.

Although Page (1987) showed preliminary evidence that aggregate formation was controlled by tree roots in the podzolic B horizons, little work has since been done on the influence of tree roots on soil structure.

On the other hand, classical theories which address homogeneous porous materials can be characterized by conventional porosity, usually measured at a definite scale, while scaling theory (Miller and Miller, 1956) or fractal approaches (Mandelbrot, 1982) naturally involve a whole spectrum of scales (self-similarity concept).

Compared to conventional porosity, the major advantage of these two self-similar structural approaches is to give a unified framework of soil structure by maintaining a continuity between observations obtained at various length scales.

Furthermore, porous media are considered to be homogeneous using scaling theory, while irregular and disordered structures occurring in nature, and particularly in soils, can be described using fractal geometry (the word "fractal" comes from the Latin adjective "fractus", which means irregular).

The recent use of fractal geometry (Mandelbrot, 1982, 1984; Feder, 1988) for the study of soil structure (Ahl and Niemeyer, 1989; Bartoli et al., 1991a; Young and Crawford, 1991; Bartoli et al., 1992a, b), soil fragmentation (Tyler and Wheatcraft, 1989; Bartoli et al., 1991a; Rieu and Sposito, 1991a, b; Perfect and Kay, 1991) and soil water properties (Tyler and Wheatcraft, 1989, 1990a, b; Toledo et al., 1990; Rieu and Sposito, 1991a, b) has opened new windows to the study of structural heterogeneities, soil water properties and underlying soil aggregation mechanisms.

It is the aim of the present paper to combine the approaches of colloidal

chemistry and fractal geometry to study aggregate structures of the A_1 (0–10 cm) and A_1 (B) (20–30 cm) horizons of an acid brown soil along a 23 meter transect under three fir trees, in order to test the hypothesis that the root zone of fir trees affects the soil structure.

MATERIALS AND METHODS

Soil

The study was conducted on a sandy acid brown soil (Typic Cryochrept) developed in Triassic intermediate sandstone at Mortagne forest in the Vosges Mountains, eastern France (altitude: 600 m, annual rainfall: 1100 mm).

The soil had a mull horizon (C/N ratio: 18 to 22) and supports a forest of about hundred year old beeches (*Fagus silvatica*: 10%) and firs (*Abies pectinata*: 90%) which were recently affected by the forest decline syndrome (the needle deficit was larger than 25%).

Sampling

In May 1986, soil samples were systematically collected every meter and at two depths 0–10 cm (A_1 horizon) and 20–30 cm (A_1 (B) horizon) along a 23 m transect under three fir trees.

Soil sampling was carried out using the Eijkelpamp–Giesbeek 07-Set C 84 kit for soft and hard soils with Edelman and Riverside augers, a closed ring holder for 8 cm diameter and 5 cm high rings.

The presence of fir roots inside or just near the 250 cm³ soil core samples allowed us to demarcate root zones as shown in Figs. 4, 5 and 7.

Aggregate structure (mercury porosimetry) and water-stability, as well as standard physico-chemical and ultramicro-morphology analyses, were carried out on < 2 mm air-dried soil samples.

Standard physico-chemical analyses

These analyses were:

- (i) Organic carbon determination by CHON Carlo Erba gas analyser.
- (ii) Carbohydrate determination on some selected samples following the procedure described by Cheshire (1979) and Spiteller (1980): a two-gram soil sample was hydrolysed with H₂SO₄, the monomeric sugars contained in the hydrolysates were then reduced to alditols and acetylated to aditol acetates before gas chromatography analysis.
- (iii) Determination of poorly-ordered Al and Fe hydrous oxides by chemical dissolution with the oxalic acid/ammonium oxalate buffer (ox) of Tamm

at pH 3 and 20°C for 4 hours in the absence of light (ratio 1:40) (Schwertmann, 1964).

(iv) Determination of exchangeable cations by atomic absorption spectrometry after one hour 0.5M NH_4Cl exchange (ratio 1:40),

(v) Particle size distribution following NaOCl pretreatment – Na resin method described by Rouiller et al. (1972, 1974).

(vi) Soil surface charges by continuous potentiometric titrations in a N_2 atmosphere using a 0.5 g:50 ml soil:solution ratio with an automatic DL 25 Mettler titrator linked to a Mackintosh SE microprocessor; the zero point of charge (ZPC) was obtained graphically as the intercept of the net amount of surface charges (σ) vs pH curves. At pH 3, which was the acidic end of the titration process, soil suspensions were centrifuged at 33,000g for 30 min. Determinations of Al by atomic absorption spectrometry and organic C by TCM 1104 Carlo Erba analyser were performed on the supernatants.

Aggregate water-stability

Aggregate water-stability tests were carried out according to the method described by Bartoli et al. (1991b).

A 2 g sample was added to 200 ml distilled water in a sieving machine with nine brass sieves and 6 hours was an appropriate time of disaggregation in water because kinetics of soil disaggregation were slower from that time of wet-sieving (Bartoli et al., 1991a). Relative error on aggregates and coarse sands remaining on the sieve was 2–8% (triplicates). Water-stable $> 200 \mu\text{m}$ aggregates were calculated by subtracting the $> 200 \mu\text{m}$ fraction obtained after the strongest disaggregation (Na-resin) from the fraction remaining on the sieve after 6 h of shaking.

Fractal geometry and mercury porosimetry

In order to use fractal geometry (Mandelbrot, 1982, 1984; Feder, 1988) to describe soil structures, it will be helpful first to summarize how the fractal approach has recently been introduced to the study of irregular porous materials. Soils, and more generally porous materials, are characterized by their mass, m , porosity, p , and mass-pore interface, s .

The first property of a fractal object is its ability to be described in terms of dilatational self-similarity (heterogeneity is independant of scale). Following Van Damme and co-workers (Van Damme et al., 1988; Ben Ohoud, 1988, Van Damme and Ben Ohoud, 1990) and Pfeifer and Obert (1989), it is possible to look for simple fractal power relationships between the three bulk properties m , p and s of the material studied and the square side or circle radius, R , in which the material can be characterized:

$$m(R) = R^{D_m} \quad (1)$$

$$p(R) = R^{D_p} \quad (2)$$

$$s(R) = R^{D_s} \quad (3)$$

where D_m , D_p and D_s are the mass fractal, pore fractal and surface fractal dimensions respectively.

If $D_m = D_p = D_s = 3$, the material is non-fractal. This is the case for homogeneous regular three-dimensional porous aggregates such as silica gel (Avnir et al., 1985) or kaolinite (van Damme and Ben Ohoud, 1990). The dimension D of a fractal object is not an integer as it is in Euclidean geometry, but always a fraction; for example, the dimension of a fractal crumpled plane is between the Euclidean dimension of a plane (2 for a completely smooth surface) and that of a volume (3 for an infinitely crumpled plane).

The following examples illustrate that complex porous media may be represented by simple fractal scaling models having similar mass fractal dimensions.

The ideal Sierpinski carpet in Fig. 1a has a fractal dimension of 1.89. The statistically self-similar Sierpinski carpet (Fig. 1b after Kaye, 1989) or "Swiss cheese model" (Fig. 1c after Mandelbrot, 1984) of the same fractal dimension, with either squared or circled holes randomized, has interconnecting pores as in the $A_1(B)$ horizon studied (Fig. 2).

Figures 1 and 2 show that sections with vastly different structures can have similar values for the mass fractal dimensions. In order to discriminate these structures it is therefore necessary to use other complementary fractal dimensions such as the surface fractal dimension D_s or the spreading dimension which is representative of the connectivity of the fractal (Jacquin and Adler, 1987).

The classical way of calculating a surface fractal dimension is image analysis by following the soil-pore interface with a yardstick of different scales (Fig. 3a). This Hausdorff-Besicovitch dimension (Mandelbrot, 1982, 1984; Feder, 1988) is the sensu stricto fractal dimension because a fractal object can be defined as an aggregate characterized by a Hausdorff-Besicovitch dimension strictly ranked above its topological dimension.

The other way for following interfaces is much more indirect by using a surface probe such as nitrogen (Avnir et al., 1985; Ben Ohoud, 1988; Van Damme and Ben Ohoud, 1990) or mercury (Friesen and Mikula, 1987; Bartoli et al., 1991a, 1992a, b). In these cases, the surface fractal dimension D_s is only a similitude fractal dimension because the surface probe mostly describe the surface accessible by the probe (Fig. 3b) and not the real surface (Fig. 3a).

Surface accessibility may also vary with the scale and the probe-type used. For example, a large pore to which access from the surface can be gained by

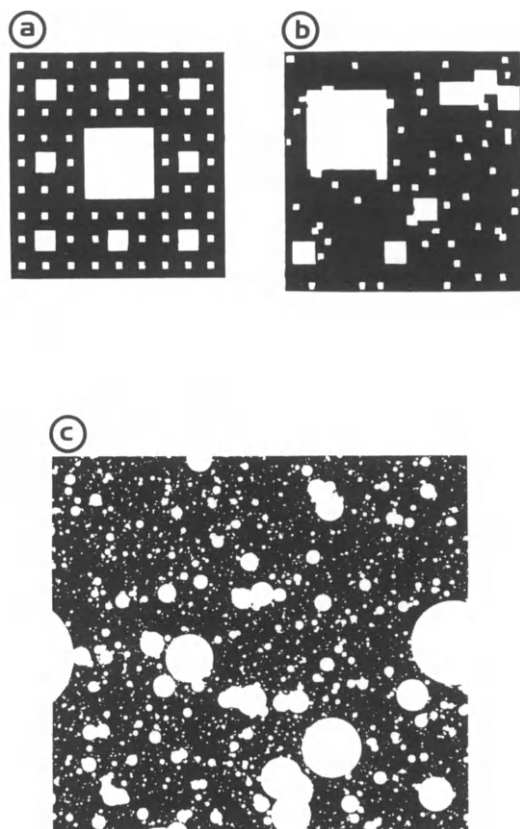


Fig. 1. (a) Ideal Sierpinski carpet of fractal dimension 1.89 and (b) statistically self-similar third-order Sierpinski carpet of the same fractal structure as that illustrated in (a) (after Kaye, 1989). (c) "Swiss cheese model" of the same fractal structure ($D=1.9$) (after Mandelbrot, 1984).

mercury only through a narrow channel will be counted as a relatively small pore (Fig. 3b).

However, a positive relationship may exist between the Hausdorff–Besicovitch dimension and the similitude surface fractal dimension D_s . Measurements of both dimensions on the same soil samples would be strongly recommended in future to develop the fractal approach to the study of soil structure.

Compared to the N_2 –BET method, mercury porosimetry as an aggregate-structure probe has the disadvantage to follow soil–pore interfaces with poorer resolution (pore neck effects) but the advantage is that a wider range of sizes may be probed. Compared to image analysis techniques, mercury experiments cost little time.

A Carlo Erba 2000 mercury depression and intrusion porosimeter, linked

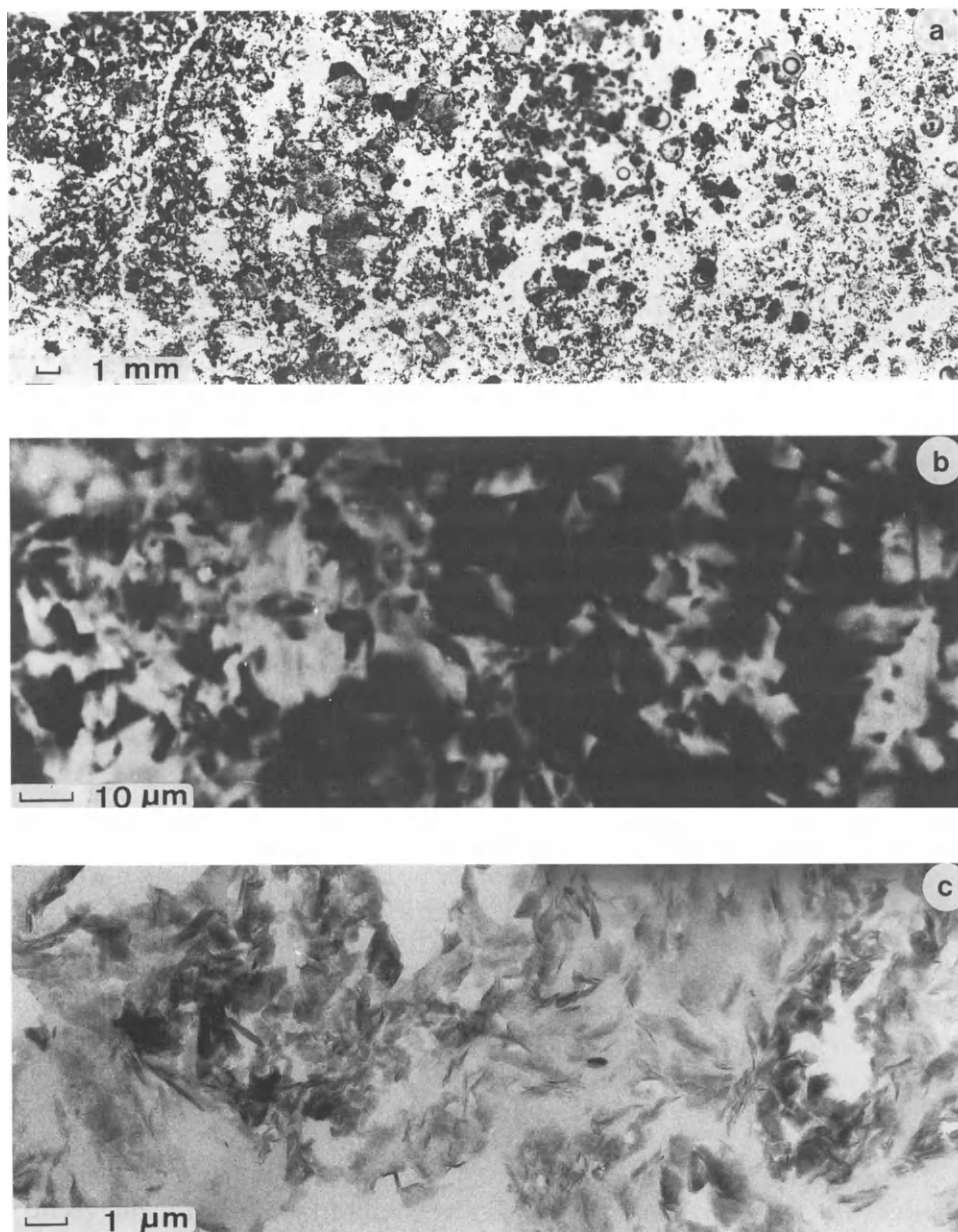


Fig. 2. (a) Contact thin soil section, (b) backscattered SEM little thin soil section and (c) TEM ultra-thin soil section micrographs of the A₁(B) sandy acid brown soil (mass fractal dimension $D=1.95$) (after Bartoli et al., 1991a).

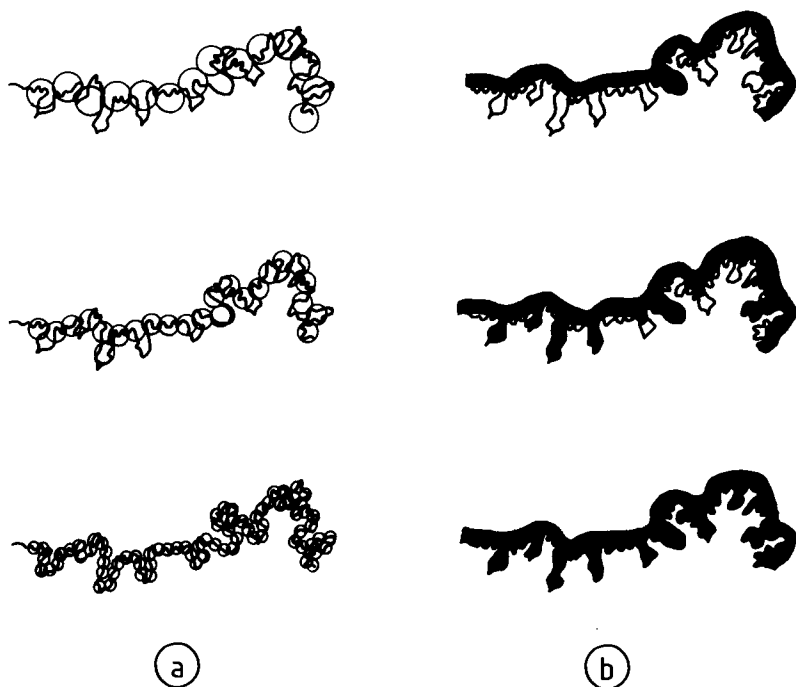


Fig. 3. Exploration of soil-pore interface, presented here in two dimensions, (a) with an image analysis yardstick of different scales, by following the interface (Hausdorff-Besicovitch dimension) and (b) with mercury as a scale-probe, by filling the pores as an inverse function of their radius (surface fractal dimension D_s).

to a Carlo Erba CUT/HEC 960 computer, was used to determine the distribution of pore radii from 4 to 10^6 nm using 1 g < 2 mm air-dried soil sample previously outgassed at room temperature for 16 hours. A value for the surface tension of mercury of 0.48 N m^{-1} and a contact angle of 141° were used with the Laplace equation assuming cylindrical pores in the calculation.

The surface fractal dimensions D_s were calculated from the mercury porosimetry data using the Sierpinski cube self-similar structure, which is also called the Menger sponge (Mandelbrot, 1982; Friesen and Mukula, 1987; Kaye, 1989).

The self-similar algorithm used in this paper was previously suggested by Pfeifer (1984), further described by Friesen and Mikula (1987) and recently introduced in soil science by Bartoli et al. (1991a):

$$\Delta V_p / \Delta R \propto R^{(2-D_s)} \quad (4)$$

where V_p is the pore volume, R the average pore radius and D_s the fractal dimension of the surface which can be therefore deduced from the slope of the linear log-log plot of $\Delta V_p / \Delta R$ versus R .

We recently demonstrated that the $A_1(B)$ (12–17 cm) horizon studied here, was not a pore fractal but a mass fractal ($D_m=1.95$ in two dimensions) in the 10^{-2} to 10^{-6} μm scale-range studied by image analysis on thin, very-thin and ultra-thin soil sections (Fig. 2; Bartoli et al., 1991a). $A_1(B)$ aggregates were also surface fractal ($D_s=2.75$ in three dimensions), that is to say were both irregular and self-similar, as determined by mercury porosimetry (Bartoli et al., 1991a).

The main objective of the present paper is to study the spatial variability of this soil structure and heterogeneity index along the 23 m transect under three fir trees in order to test the hypothesis that the root zone of fir trees affects soil structure.

Statistics

Statistics were carried out using the Statgraphics program package (version 5; STSC's software products) into an IBM PC computer. The range test used here was the least significant differences (LSD). Small significance levels (less than 0.05) indicate that the response variable differs significantly across the levels of the classification factor.

RESULTS AND DISCUSSION

Soil texture, constituents and surface charges

The soil silt and clay fractions varied a little spatially along the 23 m transect for both the A_1 and the $A_1(B)$ horizons (Fig. 4) which had on average the same soil texture (coarse sand 0.2–2 mm = 46.9 ± 3.9 , fine sand 50–200 μm = 27.4 ± 2.9 , coarse silt 20–50 μm = 5.1 ± 1.0 , fine silt 2–20 μm = 9.1 ± 0.7 , clays = 11.2 ± 1.7 for the 8 A_1 and 8 $A_1(B)$ soil samples studied).

From chemical and X-ray data, the silty and sandy fractions contained about 85% quartz, 15% orthoclase and 0.5% plagioclase; the clay fraction was mainly illite, illite-vermiculite interstratified clays and kaolinite.

In contrast, amounts of both organic carbon and Al_{ox} clearly discriminate between the two soil horizons studied, the A_1 horizon being more organic and less rich in poorly-ordered Al hydrous oxides than the $A_1(B)$ one (Figs. 5 and 8; least significant differences $\text{LSD}=0.0019$ and 0.0004 for organic C and Al_{ox} respectively).

Consequently the Al_{ox} –organic C correlation was significantly negative ($r=-0.61$; $P<0.05$ for the 16 samples studied).

The tendency to have, for each horizon, three organic maxima and three Al_{ox} , pH 3-end titration Al minima corresponding to the three fir root zones (Figs. 5 and 8) was not statistically significant ($\text{LSD}=0.7897$ and 0.6523 for Al_{ox} and organic C respectively).

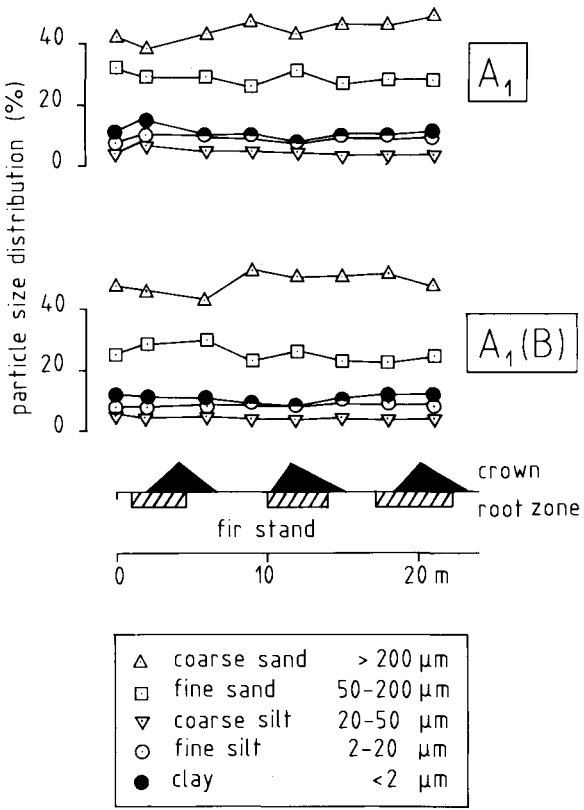


Fig. 4. Spatial distribution of particle size classes along the 23 m transect under 3 fir trees.

On the other hand, it was possible to detect, in the root zone, polysaccharides from both plant and microbial origin, their galactose+mannose/arabinose+xylose ratio (1.1 to 1.3) being between 0.5 and 2 (Murayama, 1981; Oades, 1984; Baldock et al., 1987). The contribution to soil organic matter from the fir root zone may be directly from the root material itself and its decomposition and/or indirectly from stimulation of microbial activity in the rhizosphere.

From some carbohydrate analyses we also found that the A₁₁ (0–5 cm) and A₁₂ (5–10 cm) horizons collected in the fir root zone contained about 2 to 3 more amounts of polysaccharides than the corresponding non-root zone horizons.

Finally, Fig. 6 indicates that (i) the chemical buffer of the soil studied was low and (ii) the zero point of charge (ZPC) decreased and the σ -pH curve slopes increased as a function of organic matter, from the A₁ to the A₁(B) horizon, as previously observed in Oxisols (Gallez et al., 1976; Gillman and Bell, 1976; Dixit, 1979), andosols (Iniguez and Val, 1982; Radcliffe and

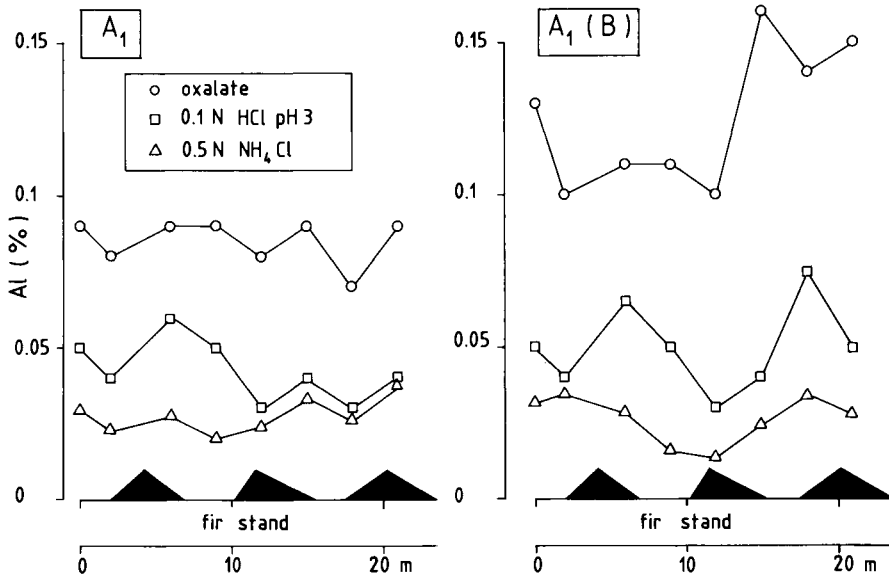


Fig. 5. Spatial distribution of poorly-ordered Al hydrous oxides and exchangeable Al along the 23 m transect under 3 fir trees.

Gillman, 1985) or temperate and cold soils (Laverdiere et al., 1977; Ross, 1980; Bartoli and Philippy, 1989).

It also appeared that the A_1 horizon was negatively charged (zero point of titration $ZPT - ZPC = -0.2$ to 1 meq/100 g) whereas the $A_1(B)$ horizon was almost uncharged. Zero point of charge also clearly discriminates the two soil horizons studied, the ZPC of the A_1 horizon being lower than the ZPC of the $A_1(B)$ one (Fig. 6; $LSD = 0.0000$).

Similarly, the following multiple regression suggests that the soil zero point of charge was mainly controlled by both positively charged poorly-ordered Al hydrous oxides and by negatively charged organic materials (electrostatic bonds):

$$ZPC = 7.30Al_{ox}(\%) - 0.14org.C(\%) + 3.69 \quad (r=0.85; P<0.001)$$

Aggregate water-stability

A clear distinction between the two horizons studied was again observed with many more water-stable aggregates in the organic A_1 horizon than in the $A_1(B)$ (Fig. 8; $LSD = 0.0000$).

Consequently, positive correlation between the amount of water-stable aggregates and organic carbon content was established ($r=0.83$; $P<0.001$) confirming numerous previous studies (i.e. Tisdall and Oades, 1982; Chaney and Swift, 1984; Douglas et al., 1986; Bartoli et al., 1988).

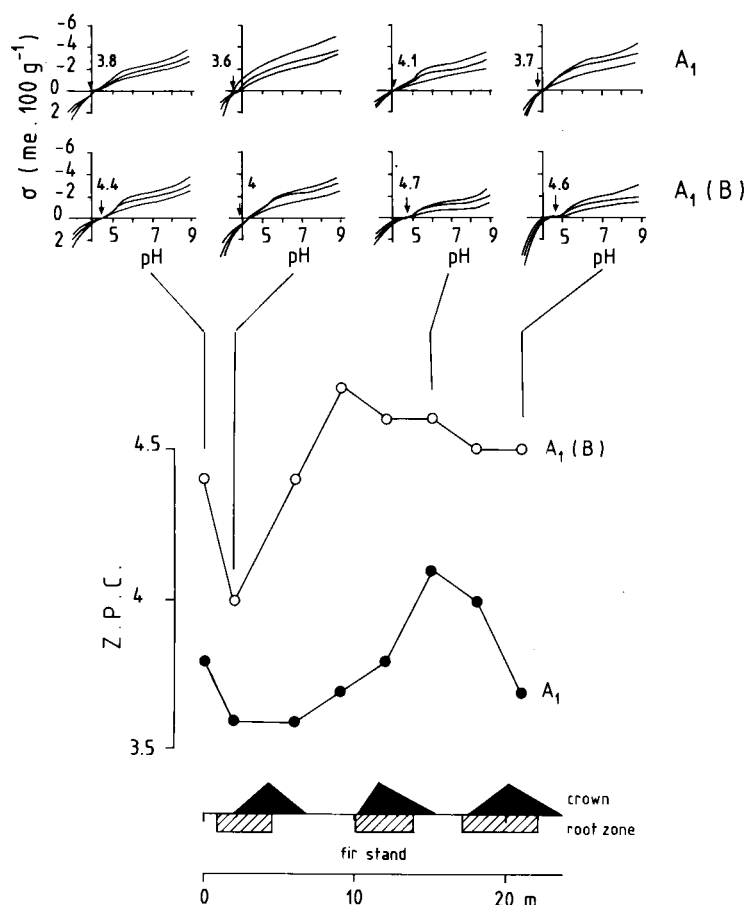


Fig. 6. Spatial distribution of zero point of charge along the 23 m transect under 3 fir trees with some representative surface charges as a function of pH and ionic strength curves.

Although the fir root zone effect on aggregate stability measured in this study was statistically non-significant ($\text{LSD}=0.3432$ and 0.5658 for the A_1 and $A_1(B)$ horizons, respectively) the fir root zone polysaccharides, from both plant and microbial origin, would play an important role in this aggregate stabilization, as it was often demonstrated for soil polysaccharides (Tisdall and Oades, 1982; McKeague et al., 1986). The probable reason for not detecting the effect here is the small amount of samples.

Aggregate structure and fractal geometry

The mercury porosity curves of the sandy soils studied, with most of the pore volume (0.2 to $0.4 \text{ cm}^3 \text{ g}^{-1}$) at a pore radius of $2\text{--}30 \mu\text{m}$, were similar to the previous porosity curves of this $A_1(B)$ acid brown soil (Bartoli et al.,

1991a) or other similar sandy soils with less than 15% clays (Nagpal et al., 1972; Chretien, 1987).

Porosities, pore size distributions, and probably pore shapes, are rather similar in these aggregates confirming the usefulness of other soil structure indexes such as the surface fractal dimension D_s .

Two domains can be distinguished from the log-log $\Delta V/\Delta R$ vs R curves (Fig. 7).

The first and predominant domain, which mainly corresponds to the intra-aggregate pore systems, followed the laws of fractal geometry, the validity of eq. (4) being confirmed. In each soil horizon studied, the upper limit/lower limit ratio of the aggregate fractal regime largely exceeds $2^{1/D_s}$, which is the minimal condition to accept an experimental D_s value over a range of fractal self-similarity (Pfeifer and Obert, 1989).

In contrast, a second domain can be defined, particularly in the $A_1(B)$ soil samples where a shoulder, or a beginning of a shoulder, centered at 10–30 μm pore radius occurs. This may be attributed either to an artefact due to a certain degree of order in inter-millimetric aggregate fabric or to more regular structures at larger scales.

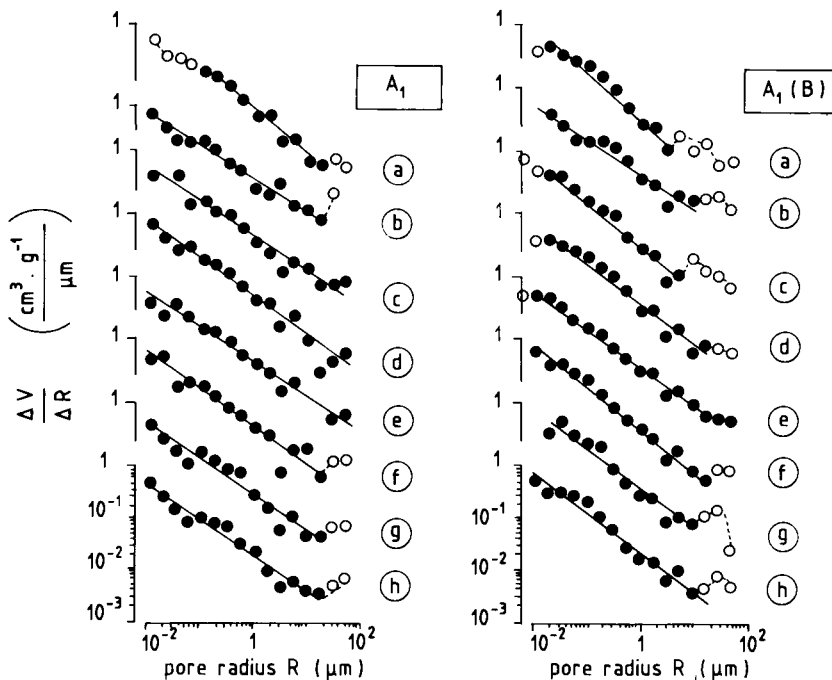


Fig. 7. Relationships between $\Delta V/\Delta R$ and mean pore radius R using the fractal Sierpinski cube model of Friesen and Mikula (1987). Millimetric aggregate samples *a* to *h* were collected from 0 to 21 m along the transect as shown in Figs. 4, 5 and 8.

As in the temperate silty and sandy soils recently studied by Bartoli et al. (1991a), the focal point is the repetition of disorder at all length scales, the repetition of structure within structure. Relative flexibility of clays (mainly illites, illite-vermiculites associated with kaolinites) which occurred in these temperate soils would explain this large degree of disorder.

Similarly, Wong and Howard (1986) suggested that the fractal properties of the pore surfaces of the sandstones and shales are due to the presence of fractal flexible clay minerals on pore surfaces of these rocks; in contrast the pore surfaces of limestones, free from clay, were smooth ($D_s=2$) on length scales larger than about 5 nm.

The upper limits to the length scale of the self-similar regions varied from 2.5 to 45 μm (Fig. 7). Similarly, using SEM image analysis, Katz and Thomp-

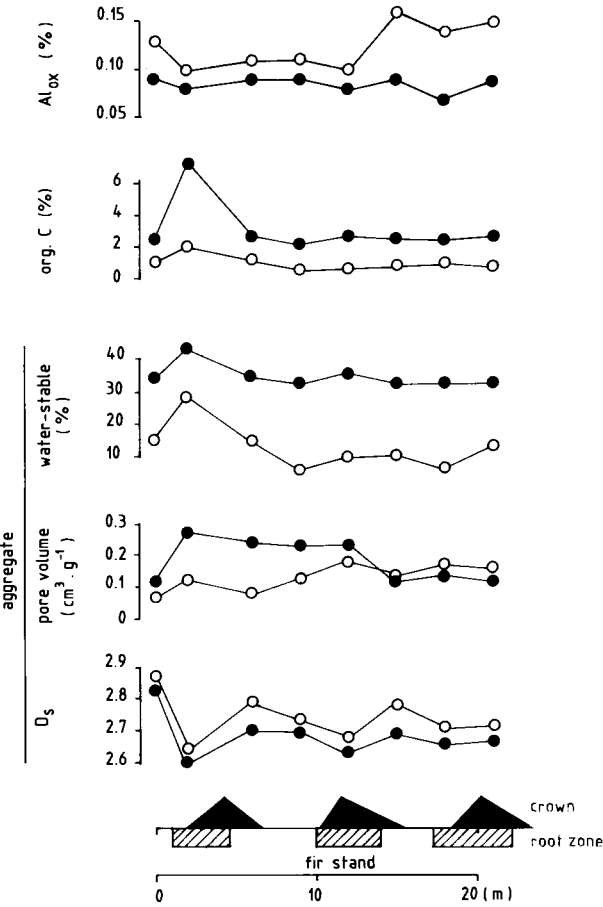


Fig. 8. Spatial distribution of aggregate chemical and structural characteristics along the 23 m transect under 3 fir trees. (●) = A₁ horizon and (○) = A₁(B) horizon.

son (1985) and Krohn and Thompson (1986) have measured the fractal dimension of the pore-rock interface for some fractured sandstones, shales and carbonates and report surface fractal dimension values ranging from 2.3 to 2.9 with upper limits of fractal behaviour of 2 to 98 μm .

Although it was not statistically quantified, Fig. 7 also indicates that the fractal domain had often a larger range of spatial scales in the upper less dense organic A_1 horizon (up to 14–45 μm) than in the deeper Al-rich A_1 (B) horizon (up to 2.5–14 μm). Similarly, Vacher and Courtens (1990) had recently observed that the scale domain of fractal self-similarity increased in aerogels as an inverse function of density.

The relationship which exist between D_s and total fractal pore volume ($r = -0.63$; $P < 0.05$) confirmed that D_s is an intrinsic measurement for the degree of pore volume filling of the different pores (Mandelbrot, 1982, 1984; Friesen and Mikula, 1987; Pfeifer and Obert, 1989; Ahl and Niemeyer, 1989; Bartoli et al., 1992a, b).

Bottero and co-workers have demonstrated that a decrease of surface net charges of poorly-ordered Al hydrous oxide suspensions by electrostatic bonds leads to an increase of their fractal dimensions (Bottero and Bersillon, 1989).

Similarly, the formation of more compact aggregate structures in the A_1 (B) horizon (Fig. 8) would be attributed to the electrostatic attraction between poorly-ordered Al hydrous oxides and organic materials of this horizon (see the previous soil surface charges paragraph).

Finally the surface fractal dimensions D_s were not significantly different between the organic A_1 horizon and the deeper Al-rich A_1 (B) one ($\text{LSD} = 0.1384$) whereas they were smaller, for each horizon, in the non-root zone soils rather than in the root zone ones (Fig. 8; $\text{LSD} = 0.0508$ and 0.0217 for the A_1 and A_1 (B) horizons, respectively).

CONCLUSION

This study shows that in the soil samples studied when there was more organic matter and less poorly-ordered Al hydrous oxides (A_1 horizon), the soil aggregates were (i) more porous and (ii) more water-stable (Fig. 8).

Electrostatic bonds between poorly-ordered Al hydrous oxides and organic materials would possibly explain the more compact A_1 (B) aggregate structures.

The fractal domain had often a larger range of spatial scales in the A_1 horizon than in the deeper Al-rich A_1 (B) horizon.

Finally, in the sandy soils studied, soil structure was characterized by variations in heterogeneities and fractal scaling laws along a 23 m transect under 3 fir trees. The main benefit of using fractal geometry is that the surface fractal dimension D_s is a better discriminator than porosity between root zone and non-root zone soils (Fig. 8). For each horizon studied here, the aggregate

pore volume was not significantly larger for the root zone soils than the non-root zone ones whereas the surface fractal dimension D_s was significantly smaller (p less or equal to 0.05) for the root zone soils than the non-root zone soils.

The large range of fir root diameters, strengths and flexibilities would explain this larger degree of soil structure disorder. On the contrary, soil aggregate porosity, which was only a little modified by the mechanical effects of fir roots, would be an intrinsic soil property controlled by its constituents such as organic materials.

Fractal geometry may therefore provide one key to a more complete description of aggregate structure in the root zone and in the non-root zone soils. The ultimate goal would be the incorporation of the surface fractal dimensions and/or other characteristic dimensions of the fractal soil structure (spatial soil organization) into modelisations of water and nutrient cycling and/or microbial dynamics in the rhizosphere, at the soil-plant interface.

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A comparison of methods for measuring water-stable aggregates: implications for determining environmental effects on soil structure

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ABSTRACT

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This paper describes the effects of different pretreatment conditions and wet-sieving procedures on water-stable aggregate distributions of sandy and clayey textured soils (Cecil, Pacolet, and Hiwassee Series) from the Piedmont of the southern Appalachian mountains of Georgia, USA. Four soil pretreatment procedures were compared: (1) air-dried, capillary wetted (AD-CW), (2) air-dried, tension wetted (AD-TW), (3) air-dried, slaked (AD-SL), and (4) field-moist, capillary wetted (FM-CW). Air-drying soils resulted in a greater quantity of aggregates in the coarser fractions ($> 250 \mu\text{m}$), as compared to field-moist soils, with a consequent reduction in the finer fractions ($< 250 \mu\text{m}$). Differences between methods of wetting air-dried soils were more pronounced for the clayey soils where both AD-CW and AD-SL resulted in a greater proportion (22–24%) of the soil mass in the finer fractions ($< 250 \mu\text{m}$), as compared with AD-TW (4.5%). The FM-CW procedure had the lowest coefficients of variation (2–8%) for repeated measurements.

The AD-CW and FM-CW procedures were also used to compare the effects of cropping systems [conventional tillage (CT) (soybean/fallow), CT (sorghum/fallow), and no-tillage (NT) (sorghum/clover)], erosion classes (slight, moderate, and severe) and irrigation (drip-irrigated or nonirrigated) on water-stable aggregates ($> 250 \mu\text{m}$). In general, water-stable aggregates increased with decreasing intensity of cultivation, increasing severity of erosion and irrigation. Air-drying soils resulted in less differences in water-stable aggregates between treatments, but provided more detailed information on the interactive effects of cropping system and irrigation as compared with the field-moist condition. Similar differences were observed in aggregate disruption rates that were calculated from the aggregates recovered after wet-sieving for intervals of 1–32 min. Although water-stable aggregates were lower by FM-CW, this procedure showed greater separation of treatment means (e.g. erosion classes) than the AD-CW procedure.

We also compared single versus multiple-sieve techniques for describing the effects of pretreatment conditions on aggregate distributions. For the clayey Pacolet soil, a fine fractionation into eleven aggregate size classes revealed the greatest differences ($P < 0.05$) between the FM-CW and AD-CW procedures, while a coarse fractionation into macro- ($> 250 \mu\text{m}$) and micro- ($< 250 \mu\text{m}$) aggregates

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showed no differences. However, for the sandy Hiwassee soil differences in aggregate distributions between the FM-CW and AD-CW procedures were found at most levels of fractionation, but were not detected by comparing the calculated mean weighted diameters. In general, our findings emphasize the value of comparing soil specific responses to different pretreatment conditions, particularly those that compare the distributions of aggregates among size classes, as a means for describing environmental influences on soil structure.

INTRODUCTION

The size, quantity and stability of aggregates recovered from soils reflect an environmental conditioning that includes factors which enhance the aggregation of soil particles (e.g. wet-dry cycles, organic matter amendments) and those that cause their disruption (e.g. soil cultivation, bioturbation). The composition, strength and persistence of the various binding agents responsible for the formation and stabilization of soil aggregates have been discussed in many research and review articles (Harris et al., 1966; Edwards and Bremner, 1967; Tisdall and Oades, 1982; Lynch and Bragg, 1985). However, the measurement of stable soil aggregates depends on both the forces that bind particles together and the nature and magnitude of the disruptive forces applied. Further, just as the environmental history of a soil influences the size distribution and stability of aggregates so can the conditions imposed during soil sampling, preparation and analysis.

Kemper and colleagues (e.g. Kemper and Koch, 1966; Kemper and Rosenau, 1986) recognized the significance of these factors and proposed a standard procedure for measuring aggregate stability. Their method calls for air-drying and rehumidifying soil samples prior to wet-sieving to determine the recovery of aggregated particles on a single (250 μm mesh) sieve. This general procedure has been applied to a broad range of experimental conditions. Increasingly, modifications to the standard methodologies are being used to study the structural stability of soils and the nature of their aggregation. Elliott (1986) used gentle misting and slaking of soils prior to wet-sieving as a means of contrasting differences in aggregate distributions under different management histories. Other studies have capitalized on the susceptibility of soils to disruption with various chemical dispersing agents, such as periodate and sodium pyrophosphate (Tisdall and Oades, 1979), or with different degrees of physical disruption, such as ultrasonic dispersion (North, 1976; Gregorich et al., 1989), dry-sieving (Gupta and Germida, 1988) or other methods of mechanical agitation in water (Pojasok and Kay, 1990; Monrozier et al., 1991).

With the vast number of approaches being employed there is a growing need to specify the procedural variation that can be critical to the interpretation of data. The selection of a particular sample protocol, including the number and volume of samples to be collected, the tools employed, and the post-

extrication preparation (e.g. pre-sieving) and storage (e.g. temperature, water content) of samples, defines the qualitative conditions of the samples for analysis. Kemper and Koch (1966) concluded that, when these factors are standardized, the method of sample pretreatment has the greatest influence on measures of aggregation. Pretreatment conditions include the method of pre-wetting and the water content at the time of wetting. The significance of sample pretreatment will undoubtedly further depend on the wet-sieving specifications such as sample size and sieve loading, the sieve mesh diameter and the frequency and duration of sieve oscillation.

This paper explores various methods of sample pretreatment for the analysis of water-stable aggregates. Our purpose is to consider the effects of pretreatment conditions on the stability and size distribution of water-stable aggregates and the methodological implications for describing environmental effects on soil structure.

MATERIALS AND METHODS

Soils

Surface soils were sampled from four experimental sites in the piedmont of the southern Appalachian mountains near Athens, GA., USA ($33^{\circ}54'N$, $83^{\circ}24'W$). The mean annual precipitation of the area is 1270 mm and evaporation is 1564 mm. The sites were selected to provide a range of soil textures from loamy sand to sandy clay loam surfaces (Table 1). Four soils were sampled from sites 1–3. They included two soil organic carbon (SOC) aggraded fescue (*Festuca arundinacea* Schreb.) sods, a Hiwassee sandy loam (clayey,

TABLE 1

Management practices, soil series, textures, concentrations of carbon, clay and silt and erosion classes of the sites included in this study

Site	Soil management	soil series	Texture	Carbon (g/kg)	Clay (g/kg)	Silt (g/kg)	Erosion class ^a
1	Fescue Sod	Cecil	loam	24.4	130	260	slight
2	Fescue Sod	Hiwassee	sandy loam	17.8	165	120	slight
3a	No-tillage	Cecil	loamy sand	13.0	71	127	slight
3b	No-tillage	Pacolet	sandy clay loam	8.3	210	149	severe
4a	Various ^b	Cecil	loamy sand	6.7	57	150	slight
4b	Various	Cecil	sandy loam	12.1	110	210	moderate
4c	Various	Pacolet	sandy clay loam	14.0	190	220	severe

^aAccording to USDA-SCS criteria (Soil Survey Staff, 1981).

^bThree cropping systems [CT (soybean/fallow); CT (sorghum/fallow), NT (sorghum/clover)] and irrigated and non-irrigated treatments were imposed on each erosion class. Carbon contents are averages across crop culture and irrigation treatments.

kaolinitic, thermic, Rhodic Hapludult) and a Cecil loam (clayey, kaolinitic, thermic, Typic Kanhapludult); and two SOC depleted soils recently placed under no-tillage management (planted to *Sorghum bicolor* [L. Moench] and *Triticum aestivum*), a Pacolet sandy clay loam and a Cecil loamy sand (clayey, kaolinitic, thermic, Typic Kanhapludult).

Site 4 was a soil renovation experiment in which three cropping systems were imposed on three erosion classes (slight, moderate and severe) each under drip-irrigated (to maintain 0.03 MPa at 22 cm) and non-irrigated conditions for five years (Bruce et al., 1990). The three cropping systems were: (a) soybeans (*Glycine max* L. Merr.) planted into a conventionally tilled (disc-harrowed) seed bed following winter fallow [CT (soybean/fallow)], (b) grain sorghum planted into a conventionally tilled seed bed following winter fallow [CT (sorghum/fallow)], and (c) grain sorghum planted no-tillage into a crimson clover (*Trifolium incarnatum* L. Tibble) winter cover [NT (sorghum/clover)]. Each cropping system by erosion class by irrigation treatment was replicated three times. Further details on the tillage practices and experimental design are described by Bruce et al. (1990) and Langdale et al. (1990).

Soils were sampled with a thin walled, steel cylindrical corer (5.8 or 10 cm diameter) that was inserted by a steady load to the required depth. The number and size of samples for each experiment are specified below. Soils were stored field-moist (3°C) in crush-resistant air-tight containers prior to sample pretreatment. All samples were pre-sieved prior to wet-sieving to remove stones and coarse organic matter and to define the initial dimensions of the aggregates for analysis. In pre-sieving, soils were gently teased apart by hand, breaking the peds along fissures of least resistance to pass the pre-sieve openings (10 mm diameter).

Wet-sieving apparatus and specifications

Measurements of water-stable aggregates were made using an instrument similar in principle to the Yoder (1936) wet-sieving apparatus (Fig. 1). The modified apparatus was designed to handle larger masses of soil on stacked sieves (21.6 cm diameter) and to allow for complete recovery of all particle fractions from individual samples. Where multiple fractions were separated, only sieves with mesh openings $\geq 250 \mu\text{m}$ diameter were contained in the oscillation cylinders. Following wet-sieving, the cylinders were drained and particles $< 250 \mu\text{m}$ in diameter were collected on the finer sieves. To minimize losses in handling, aggregates were first dried on the sieves in a dehumidifying chamber (18°C), then transferred to beakers, oven dried (60°C) and weighed. Particles $< 53 \mu\text{m}$ in diameter were collected in a bucket, flocculated (24 h) with CaCl_2 (0.1M), the supernatant removed and the particles dried. Except where noted, aggregate masses were corrected for the dry

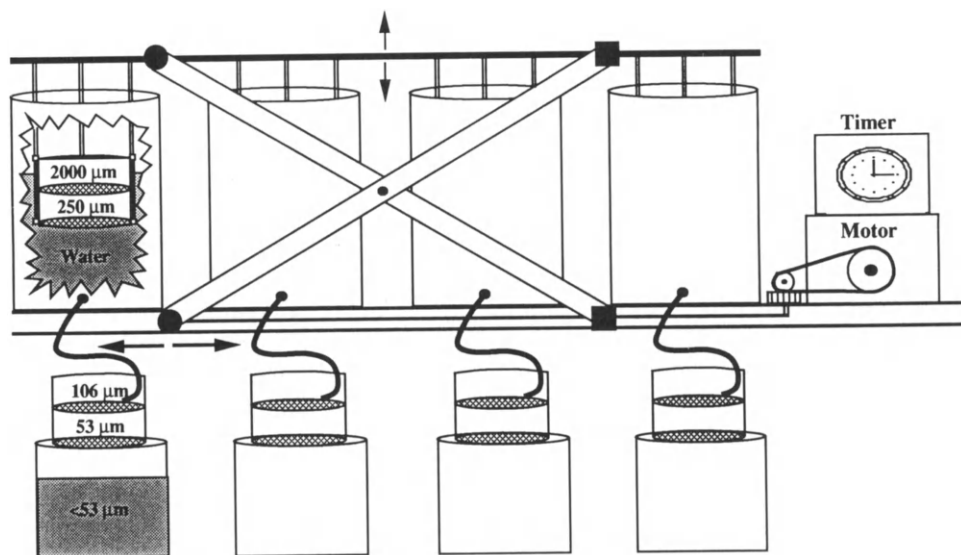


Fig. 1. Diagram of the modified Yoder (1936) wet-sieving apparatus, showing the sieving cylinders and the location of sieves $< 250 \mu\text{m}$ in diameter outside the cylinders.

weight of primary particles retained on the sieves after complete dispersion by blending in a NaOH (0.05M) solution.

The sieving specifications of oscillation time (300 s), stroke length (2 cm) and frequency (0.52 cycles/s) were held constant, except where otherwise noted. Sieve loading rates were set at between 0.40 and 0.60 g soil (dry weight equivalent)/ cm^2 . Preliminary experiments showed that loading rates lower than 0.66 g/ cm^2 gave reproducible results.

Pretreatment conditions

Four pretreatment conditions were compared: (1) air-dried and capillary wetted (AD-CW), (2) air-dried and tension wetted (AD-TW), (3) air-dried and slaked (AD-SL), and (4) field-moist and capillary wetted (FM-CW). Each soil was pre-sieved field-moist and divided in two. One half was air-dried (24°C) shortly before (ca. 72 h) pre-wetting and wet-sieving, while the other half was maintained field-moist (3°C) prior to analysis. For capillary wetting the sieves were positioned at the top of the stroke and the water level raised to form a thin film on the screen. Prewetted soils were evenly distributed over the sieve surface, allowed to wet by capillary action for 15 min and the water level raised to 1.5 cm above the sieve surface. Soils were tension wetted on the sieves by placing a moist filter paper disk between the sieve and the tension plate. The soils were wetted at constant tension (3.0 cm H_2O) for 15 min before transferring to the wet-sieving instrument. For the slaked treat-

ments, soils were transferred to dry sieves at the top of the stroke. Samples were fully immersed by rapidly lowering the sieves to the bottom of the stroke, the water level was raised to 3.5 cm above the sieve surface and sieving action was started.

Experimental comparisons

Two or more of the pretreatment procedures were applied to specific experimental conditions to (1) evaluate the effects of soil pretreatment conditions on the distributions of water-stable aggregates, (2) consider their implications for describing the effects of crop cultural practices on water-stable aggregates, (3) describe alternative ways of quantifying aggregate stabilities using single-sieve techniques, and (4) contrast the information obtained from single versus multiple-sieve estimates of soil aggregation.

RESULTS

Effects of pretreatment conditions

Multiple-sieve techniques were used to evaluate the effects of the four pretreatment conditions on water-stable aggregate distributions from soils of sites 1–3. For this experiment, 20 cores (5.8 cm in diameter) of each soil were collected to a depth of 5 cm and composited. Samples were pre-sieved and pretreated as described previously. Five aggregate size classes were collected from four replicate analyses of each soil, including aggregates >2000 , 250–2000, 106–250, 53–106 and $<53\text{ }\mu\text{m}$ in diameter. To contrast differences in particle size distributions between pretreatment conditions and across soils of different textures, the aggregate dry weights were not corrected for primary particles and their masses were expressed as a percentage of the total soil dry weight (Fig. 2). One-way analyses of variance (ANOVA) were used to compare the effects of pretreatment within each size class and soil and across soils within a size class and pretreatment. Tukey–Kramer (HSD) tests were used for separation of treatment means.

For air-dried soils, slaking (AD–SL) consistently resulted in a decrease in the proportion of soil recovered in the $>2000\text{ }\mu\text{m}$ fraction and an increase in the 250–2000 μm and sometimes the 106–250 μm fraction, particularly in the soils with higher clay content, as compared to AD–TW and AD–CW. With the exception of the Pacolet sandy clay loam soil, controlled tension wetting of air-dried soils (AD–TW) resulted in no significant ($P<0.05$) effects on the distribution of aggregated particles as compared to AD–CW soils. Relative to AD–TW, the AD–CW Pacolet soil had significant losses from the $>2000\text{ }\mu\text{m}$ fraction that resulted in significant increases in the 106–250 μm fraction. Pretreatment by AD–CW of the Pacolet soil resulted in a distribu-

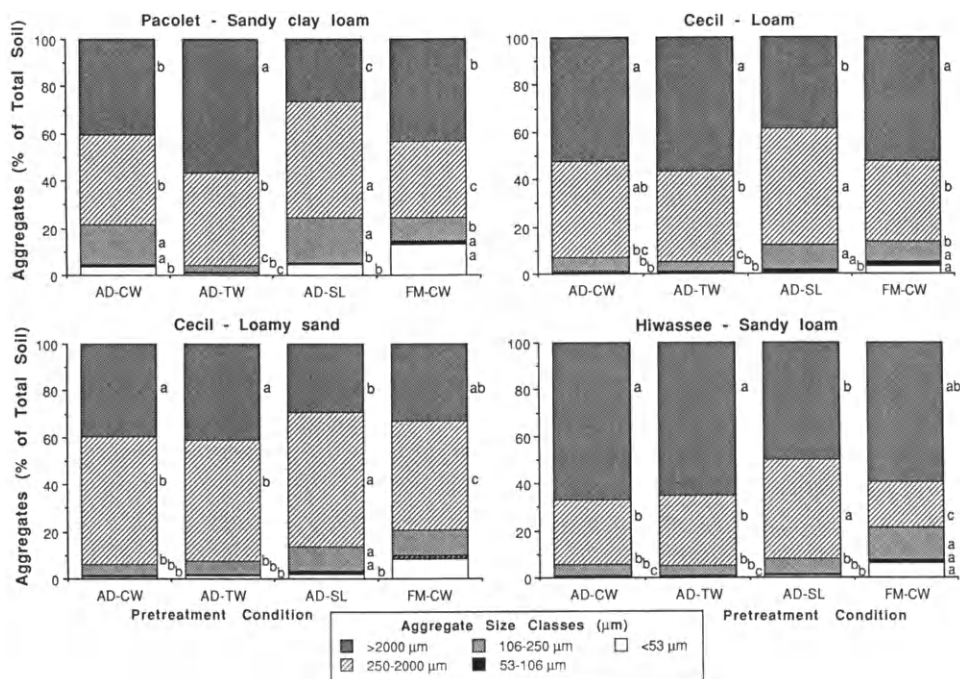


Fig. 2. Effects of the four pretreatment conditions on the distributions of water-stable aggregates from two soil organic carbon aggraded fescue sod soils (Pacolet sandy clay loam and Cecil loamy sand) and two soil organic carbon depleted tilled soils (Cecil loam and Hiwassee sandy loam). Treatment abbreviations are: air-dried capillary wetted (AD-CW), air-dried tension wetted (AD-TW), air-dried slaked (AD-SL), and field-moist capillary wetted (FM-CW). Across pretreatment conditions, the portions of the bars representing each size class that are followed by different letters are significantly different at $P < 0.05$ (ANOVA/Tukey [HSD]).

tion of aggregates which was similar to that observed for the slaked (AD-SL) soil. The Pacolet soil also had a relatively low organic carbon content and a much higher concentration of clay than the other soils in the study.

In all four soils, the FM-CW treatment resulted in a lower percentage of macroaggregates ($> 250 \mu\text{m}$) and a greater proportion of the soil mass distributed among smaller aggregate size classes as compared to AD-CW soils. The lower percentage of macroaggregates in the FM-CW could be attributed to significant ($P < 0.05$) losses from the 250–2000 μm fraction but not from the $> 2000 \mu\text{m}$ fraction. The FM-CW treatment also had consistently lower coefficients of variation (2–8%) for repeated measurements than the other pretreatment conditions (5–35%).

The percentage of macroaggregates recovered in the AD-TW treatment did not differ significantly between soils of higher [Cecil loam (95%) and Hiwassee sandy loam (95%)] and lower [Pacolet sandy clay loam (96%) and Cecil loamy sand (94%)] organic carbon content (Fig. 2). However, the two

soils with higher organic carbon contents showed greater resistance to the disruptive forces of slaking (relative to tension wetting), with 88–93% recovered as macroaggregates as compared to 78–86% for the lower organic carbon soils. Furthermore, under the FM–CW condition the higher organic carbon soils had a significantly ($P < 0.05$) greater proportion of aggregates in the $> 2000 \mu\text{m}$ fraction (52–59%) than the lower organic carbon soils (32–43%).

Pretreatment and the effects of crop cultural practices

Samples from the 10–30 mm depth of the soil renovation experiment (site 4) were used to evaluate differences in pretreatment procedures as they describe the effects of crop cultural practices on water-stable aggregates. For these analyses, single sieve ($> 250 \mu\text{m}$) determinations of water-stable aggregates were made from 25 g subsamples of soil from each replicated plot ($n = 3$). Corrections were made for the dry weights of primary particles $> 250 \mu\text{m}$

TABLE 2

The effects of cropping system, erosion class and irrigation treatments on the percentage of water-stable aggregates (%WSA) from capillary wetted air-dried (AD) and field-moist (FM) soils in comparison to other common soil properties

Crop culture treatments	Aggregates ($> 250 \mu\text{m}$)			Soil properties ¹		
	Air dried (%WSA)	Field moist (%WSA)	FM:AD ratio	Soil carbon (g/kg)	Clay (g/kg)	Infiltration rate (mm/h)
<i>Erosion class</i>						
Slight (E1)	81 ^{a 2}	39 ^c	0.48	6.7 ^b	57 ^c	35.3 ^a
Moderate (E2)	84 ^a	56 ^b	0.67	12.1 ^a	110 ^b	29.0 ^b
Severe (E3)	84 ^a	65 ^a	0.77	14.0 ^a	190 ^a	28.5 ^b
<i>Cropping system</i>						
CT (soybean/fallow)	80 ^b	47 ^b	0.59	9.4 ^b	–	23.8 ^b
CT (sorghum/fallow)	80 ^b	49 ^b	0.61	10.2 ^b	–	22.3 ^b
NT (sorghum/clover)	88 ^a	64 ^a	0.73	13.2 ^a	–	46.3 ^a
<i>Irrigation</i>						
Irrigated	87 ^a	56 ^a	0.64	11.0 ^a	–	–
Non-irrigated	79 ^b	50 ^a	0.73	10.8 ^a	–	–

¹Soil carbon was determined by dry combustion using a Leco C analyzer. Clay content was calculated from the particle size analyses using the Hydrometer method (Gee and Bauder, 1986). Infiltration rates were determined following one hour of simulated rainfall (50 mm h^{-1}) with surface residues removed (data from Bruce et al., 1990).

²For the main effects of erosion class, cropping system and irrigation level, adjusted treatment means in a given column followed by different letters are significantly different at $P < 0.05$ (ANOVA/LSD).

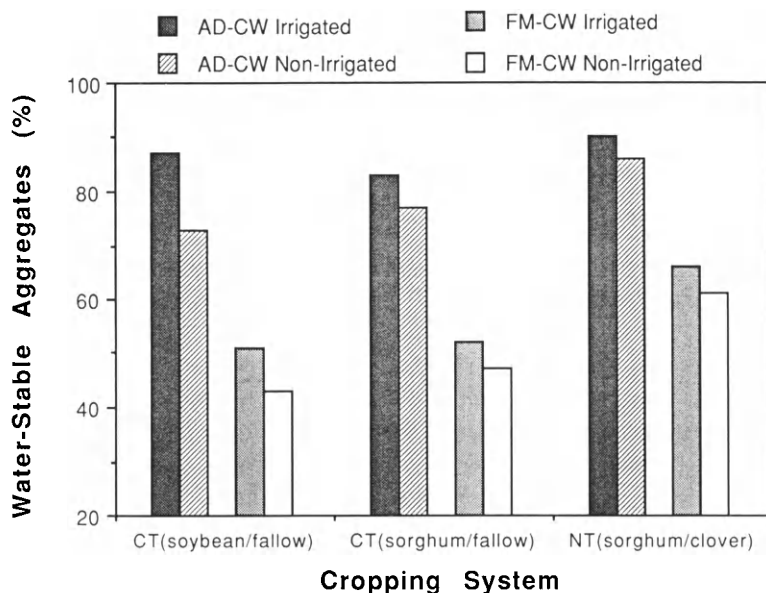


Fig. 3. Interactive effects of crop culture and irrigation on water-stable aggregates (> 250 μm) recovered following AD-CW and FM-CW pretreatment of soils from site 4. Values are adjusted treatment means ($n=3$) from the ANOVA across erosion classes.

following complete dispersion in NaOH. For each pretreatment procedure, the results were analyzed by ANOVA using least significant difference (LSD) tests for comparisons of treatment means.

The AD-CW procedure revealed relatively small but significant differences in water-stable aggregates among cropping systems and irrigation treatments but not among erosion classes (Table 2). However, there was a significant interaction of cropping system and irrigation on AD-CW water-stable aggregates ($F=7.37$, $P<0.005$). The interaction indicated that for the two conventionally tilled systems, aggregation was significantly increased by irrigation while there was no effect of irrigation in the NT (sorghum/clover) system (Fig. 3).

Although the effects of crop culture on water-stable aggregates of AD-CW soils reflected the statistical differences in soil carbon and infiltration rates, the percentage of aggregates recovered from the FM-CW soils more closely agreed with the patterns observed in the measured soil properties (Table 2). For the FM-CW procedure, water-stable aggregates increased with increasing severity of erosion and decreasing intensity of cultivation [NT (sorghum/clover) > CT (sorghum/fallow) ≥ CT (soybean/fallow)]. Although there were less water-stable aggregates by FM-CW, this procedure showed greater separation of treatment means, especially among the erosion classes, than that of the AD-CW procedure. The cropping system by irrigation interaction observed with the AD-CW procedure was not found for the FM-CW procedure.

The ratio of water-stable aggregates obtained from FM and AD soils (Table 2) indicates that differences between the procedures increased with decreasing severity of erosion and increasing intensity of cultivation [CT (soybean/fallow) > CT (sorghum/fallow) > NT (sorghum/clover)]. The smallest differences between FM-CW and AD-CW were found in the severely eroded soils of the NT (sorghum/clover) cropping system where clay and carbon content were highest.

Aggregate disruption rates

A subset of the soils from site 4 were used in oscillation time course experiments to identify appropriate durations of wet-sieving for aggregate stability analyses. For these experiments soils were first air-dried and passed through a 4.75 mm sieve to collect the aggregates retained on a 1 mm sieve. Replicate subsamples ($n=3$) of the > 1 mm aggregates were placed on a 250 μm sieve, capillary wetted and wet-sieved for intervals of 1, 2, 4, 8, 16 and 32 min. Aggregates recovered at each time point were corrected for the dry weights of primary particle (> 250 μm) recovered after dispersal in NaOH.

The duration of oscillation time during wet-sieving markedly influenced the recovery of water-stable aggregates on a single sieve (Fig. 4). The resulting aggregate disruption curves were used to calculate disruption rates as an alternative method for describing aggregate stabilities. Macro-aggregate (> 250 μm) disruption rate constants (k) were calculated using a single neg-

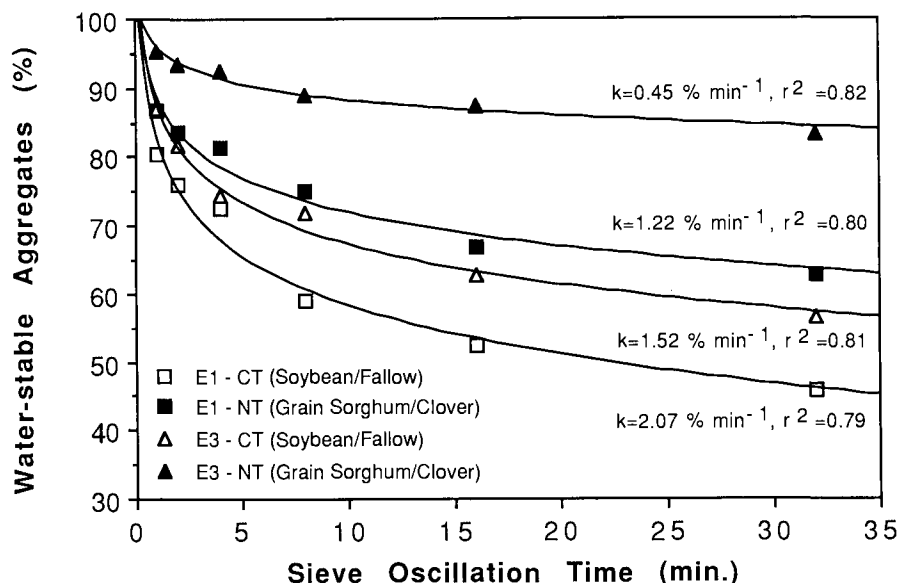


Fig. 4. Aggregate disruption curves for air-dried and capillary wetted soils of the slightly (E1) and severely (E3) eroded sites under conventional and no-tillage crop culture.

ative exponential decay model (Olson, 1963) by regressing the natural logarithm of the mean percent water-stable aggregates versus sieving time (min). A *t*-test comparison of regression slopes showed that, for slightly eroded soils (Cecil loamy sand), aggregate disruption rates (*k*) were much higher for the CT (soybean/fallow) ($2.07\% \text{ min}^{-1}$, $r^2 = 0.79$) than for NT (sorghum/clover) ($1.22\% \text{ min}^{-1}$, $r^2 = 0.79$). While aggregate disruption rates were significantly ($P < .05$) lower for the severely eroded soils, the difference in disruption rates between NT (sorghum/clover) ($0.47\% \text{ min}^{-1}$, $r^2 = 0.79$) and CT (soybean/fallow) ($1.52\% \text{ min}^{-1}$, $r^2 = 0.81$) was much greater than for the slightly eroded soils. This finding emphasizes the importance of interactions between texture and crop cultural practices in determining aggregates stabilities.

Single versus multiple size fractions

Two soils from sites 1–3 (Hiwassee and Pacolet) were used to compare the effects of pretreatment conditions on soil aggregation at different levels of fractionation. Soils were sampled and pre-sieved as described previously. Subsamples of each soil were submitted to three different levels of fractionation under the AD–CW and FM–CW pretreatments with each analysis replicated four times. These included a fine fractionation into eleven aggregate size classes ($> 4750 \mu\text{m}$, 4750–2000, 1000–2000, 500–1000, 250–500, 212–250, 150–212, 106–150, 90–106, 53–90, and $< 53 \mu\text{m}$), a medium fractionation into four aggregate size classes ($> 2000 \mu\text{m}$, 250–2000, 106–250, and $< 106 \mu\text{m}$) and a coarse fractionation into macro- ($> 250 \mu\text{m}$) and micro- ($< 250 \mu\text{m}$) aggregates only. Mean weighted diameters (MWD) were calculated from the aggregate distributions of the fine and medium level fractionations for each of the pretreatment conditions (Kemper and Rosenau, 1986). Differences between pretreatments within each size class were determined by two-tailed *t*-tests.

The fine fractionation of soils revealed considerable differences in the distributions of soil particles among the 11 aggregate size classes under the two pretreatment conditions (Fig. 5). For the Pacolet soil, the quantity of aggregates $> 4750 \mu\text{m}$ under the FM–CW condition was nearly twice that recovered from AD–CW soils. Particles lost from the $> 4750 \mu\text{m}$ fractions of the air-dried soils were largely redistributed to size classes between 90 and $500 \mu\text{m}$ in diameter. The Hiwassee soil showed a different response to the AD–CW pretreatment condition as compared to FM–CW. Air-drying resulted in a significantly greater quantity of 250 to $2000 \mu\text{m}$ aggregates and a reduction in the quantity of smaller size classes, especially the 212– $250 \mu\text{m}$ and $< 53 \mu\text{m}$ aggregates as compared to field-moist soils. The MWD of aggre-

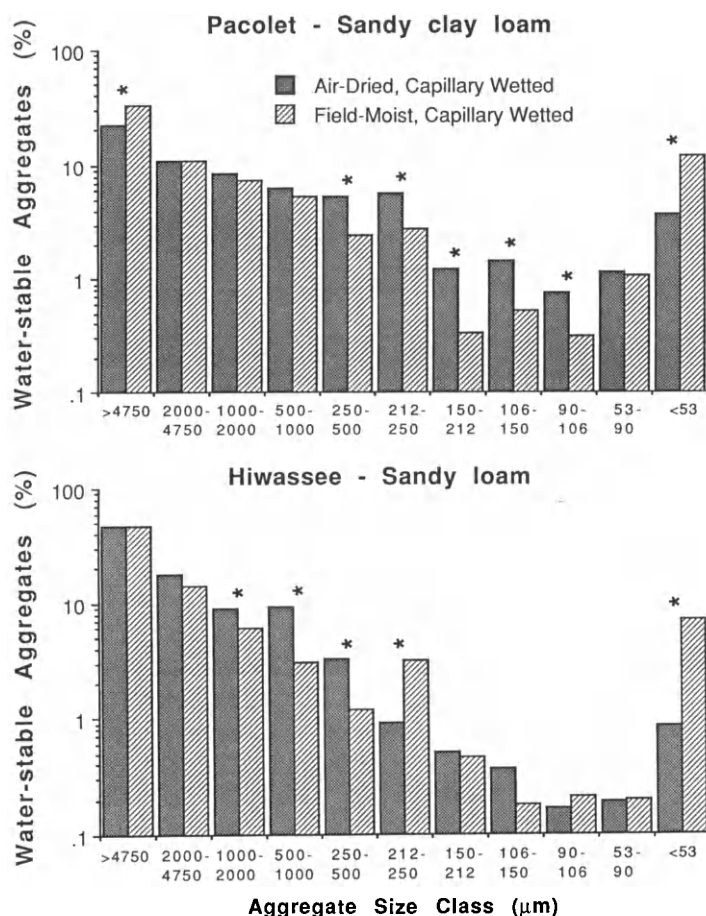


Fig. 5. Effects of air-dry and field-moist pretreatment on the distribution of water-stable aggregates from the fine fractionation of the Hiwassee and Pacolet soils. Asterisks indicate significant differences between pretreatment procedures within each size class (t -test, $P < 0.05$).

gates from the fine fractionation of the Pacolet soils was found to be significantly greater for the FM-CW than the AD-CW condition (Table 3). However, the MWD for the fine fractionation of the Hiwassee soil was not significantly affected by pretreatment conditions.

The medium and coarse level fractionations of soil aggregates yielded results that were different from the fine fractionations. For the Pacolet soil, a medium fractionation into four aggregates size classes showed significant differences between pretreatment conditions for each aggregates size class and for the MWD calculated from these data. However, for the coarse level fractionation into macro- and microaggregates, no significant differences were observed between the pretreatment conditions. A different pattern emerged for the Hiwassee soil. The MWD of the fine and medium fractionations

TABLE 3

Comparisons of water-stable aggregate distributions at three levels of fractionation for air-dried (AD-CW) and field-moist (FM-CW) soils capillary wetted before wet-sieving

Fractionation schemes and aggregate size classes (μm)	% Water-stable aggregates					
	Pacolet Sandy clay loam			Hiwassee Sandy loam		
	FM-CW		AD-CW	FM-CW		AD-CW
<i>Fine fractionation</i>						
MWD (mm) ¹	2.99	* ²	2.21	4.07	ns	4.28
<i>Medium fractionation</i>						
> 2000	58.2	*	49.3	73.8	ns	72.4
250–2000	19.8	*	30.0	12.4	*	24.3
106–250	4.7	*	12.4	4.6	ns	3.0
< 106	17.7	*	8.3	9.2	*	1.3
MWD (mm) ¹	3.73	*	3.32	4.58	ns	4.62
<i>Coarse fractionation</i>						
> 250	76.0	ns	79.6	86.1	*	96.8
< 250	24.0	ns	20.4	13.9	*	3.2

¹Aggregate mean weighted diameters (MWD) were calculated from the fine (Fig. 3) and medium fractionation data after Kemper and Rosenau (1986).

²Asterisks indicate where mean values for each soil and size class differed significantly (*t*-test, $P < 0.05$) between pretreatment procedures (AD-CW vs FM-CW).

showed no significant differences in the distribution of water-stable aggregates for the AD-CW and FM-CW pretreatments (Table 3). However, the AD-CW soils had a significantly greater proportion of aggregates in the 250–2000 μm size class and significantly less in the < 106 μm fraction. In the coarse fractionation, AD-CW soil had a significantly higher percentage of macroaggregates and significantly less microaggregates than the FM-CW soil.

DISCUSSION AND CONCLUSIONS

Our results showed that the distributions and stabilities of soil aggregates can be strongly influenced by the method of soil pretreatment. Controlled wetting of air-dried soils (AD-CW, AD-TW) tends to emphasize differences in macroaggregation, with less than 10% of the soil being recovered in the microaggregates fractions (< 250 μm). The slaked treatments had considerably lower recoveries in the > 2000 μm fractions and increases in sand sized microaggregates relative to the other air-dried treatments. Capillary wetting of field moist soils resulted in lower recoveries from the macroaggregate fractions and a greater representation of all microaggregate fractions.

In many cases a greater understanding of the forces that bind particles together can be gained by applying more than one pretreatment condition before wet-sieving. For example, in this study controlled tension wetting of the air-dried (AD-TW) clayey Pacolet soil resulted in greater recoveries of macroaggregates ($> 250 \mu\text{m}$) than in the other three soils. However, the same soil showed greater susceptibility to aggregate disruption when slaked (AD-SL) or capillary wetted (AD-CW), especially in the macroaggregate fractions. These differences would not have been apparent from a single pretreatment condition. Similarly, Dickson et al. (1991) reported that air-dried soils with higher clay contents had lower aggregate stabilities than other medium textured soils when rapidly wetted as compared to capillary wetting under high or low vacuum conditions. Other studies have shown that rapid wetting of air-dried compacted or puddled soils can generate aggregate structure through micro-cracking (Mckenzie and Dexter, 1985; Grant and Dexter, 1990).

The selection of particular pretreatment conditions for comparisons of water-stable aggregates will necessarily depend on the experimental questions. It can be useful to capitalize on soil specific responses to pretreatment as a means of describing the stabilities of soil aggregates. For example, the aggregate ratios calculated from the field moist and air-dried pretreatments of the soil renovation experiment provided a means of indexing the stabilizing influences of drying across soils that differ in texture and crop cultural history. In a separate study, Elliott (1986) used a comparison of gentle misting and slaking to describe differences in the stabilities of aggregates from native sod and cultivated soils of the North American grasslands.

The selection of a specific soil pretreatment condition can also have important consequences for describing and interpreting the effects of different environmental conditions (e.g. crop cultural histories) on water-stable aggregates. Our studies showed that measures of water-stable aggregates from soils of different cropping systems, erosion classes and irrigation treatments were significantly influenced by method of pretreatment. In general, water-stable aggregates increased with decreasing intensity of cultivation [CT (soybean/fallow) to NT (sorghum/clover)], increasing severity of erosion and irrigation. These findings were supported by the aggregate disruption rates measured on air-dried soils of the same treatments. However, air-drying soils resulted in less differences in water-stable aggregates across treatments as compared with the field-moist condition. The FM-CW procedure revealed differences in water-stable aggregates between erosion classes that were not detected with AD-CW, while air-drying soils provided information on the interactive effects of cropping system and irrigation that were not found in the field-moist condition. The interaction suggests that the greater inputs of crop biomass resulting from irrigation (Bruce et al., 1990) increased water-

stable aggregates in the conventionally tilled soils but had no significant effect in no-tillage soils where crop biomass was effected less by irrigation.

Like the drying of soils in the field, air-drying soils in the laboratory can result in precipitation of bonding agents at particle-to-particle contact points (Reid and Goss, 1982; Kemper et al., 1987) and an increase in the solid-phase cohesion of aggregated particles (Kemper and Rosenau, 1984; Bullock et al., 1988). Some of these changes in bonding forces can be partly or completely irreversible (Harris et al., 1966). Storage in an air-dried condition can also significantly increase aggregate stability (Kemper and Koch, 1966; Kemper and Rosenau, 1986). Alternatively, measurements of aggregate stability from field-moist soils can be a function of their antecedent water content (Gollany et al., 1991). The effects of water content appear to depend on factors such as organic matter and clay content, clay mineralogy and porosity (Perfect et al., 1990; Gollany et al., 1991). However, it is very difficult to separate the effects of physio-chemical factors from the biological influences that accumulate with changes in water content and both are likely to contribute to changes in aggregate stabilities (Reid and Goss, 1982; Perfect et al., 1990).

While air-drying soils establishes a uniform condition for comparing and contrasting aggregation in soils of different water contents, it may also tend to emphasize the contributions of particular binding forces while diminishing others, especially those of interest in studies of biological effects on soil structure. Gentle pre-wetting and analyzing soils immediately after field sampling avoids the effects of air-drying and storage (Gollany et al., 1991) and may prove to be the preferred approach for describing biologically mediated effects on soil structure.

A number of early studies (e.g. Schaller and Stockinger, 1953; Kemper and Koch, 1966) concluded that aggregate stability data obtained from single-sieve methods are adequate for describing relationships between soil structure and various physio-chemical properties of the soils. Others have emphasized the close correlation between single-sieve estimates of aggregate stability and mean weighted diameters (MWD) calculated from results obtained by the multiple-sieve Yoder (1936) method (Schaller and Stockinger, 1953; Panabokke and Quirk, 1957; Kemper and Koch, 1966). Our results and general experience suggest caution in accepting these observations as universal and in uniformly adopting single-sieve methodologies for describing biologically mediated effects on soil structure.

Important distinctions exist between measures of aggregate size distributions and the stabilities of specific aggregate fractions. The more conventional single-sieve determinations of aggregate stabilities describe properties of the binding agents responsible for aggregation. They reflect the composition and strength of these binding agents and are influenced by factors such as clay and organic matter content (Kemper and Koch, 1966). However, ag-

gregate stability measurements are most often made on a pre-selected size class of aggregates and, therefore, do not represent a condition of the soils as a whole. Measures of aggregate stability also depend on the size of aggregates selected for analysis (Panabokke and Quirk, 1957). These factors contribute to difficulties in interpreting results from soils where there are differences in aggregate distributions and thus the contributions of a specific size class to the whole soil. Alternatively, the distribution of aggregated particles reflects the arrangement of solids and voids within the soil. These, in turn, affect the physical properties (e.g. water infiltration, aeration) which mediate the biological and chemical processes (microbial and faunal activities, organic matter decomposition) that influence nutrient cycling and plant growth (Letey, 1985).

The level of soil fractionation can be important to identifying particular effects on soil structure. For example, our results showed that differences in the effects of capillary wetting air-dried or field-moist soils were more or less apparent at different levels of resolution. In the Pacolet soil, differences in the distributions of water-stable aggregates were observed at the fine and medium levels of fractionation that were not apparent by separating macro- and microaggregates. Differences in the aggregate distributions from the Hiwassee soil were found at all levels of fraction; however, these effects were not detected by the MWD calculations. These results suggest that the selection of a specific fractionation regime can be important to describing differences in soil structure. We recommend that investigators conduct preliminary experiments to identify fractionation regimes that are most appropriate to their experimental questions and conditions. We further recommend that results of water-stable aggregate analyses be accompanied by complete descriptions of the procedures applied and that the procedural conditions be used to qualify the interpretations of the data presented.

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Water repellency of sieve fractions from sandy soils and relationships with organic material and soil structure

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ABSTRACT

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In soil micromorphology most studies are done with light and electron microscopes on thin sections from selected samples. Information is obtained on nature, distribution, size, form, quantity, etc. of soil constituents and features. Significant data, not readily obtainable from thin sections, can be supplied by using sieve fractions. Amongst such data are specific soil structures (rounded macro-aggregates, micro-aggregates and coated plant fragments) which may, partly or wholly, have been made by soil biota.

During the study of water repellency of agricultural and uncultivated sandy soils in the southwestern Netherlands, each sample was sieved, washed and treated with hydrogen peroxide. By measuring the water drop penetration time (WDPT) of a soil sample and of its sieve fractions, and by microscopic examination of soil components and features in each of these, one can indicate which soil constituents are important to the building up of water repellency.

The appearance and degree of water repellency are closely related with organic matter (plant fragments, roots, etc.). This organic matter can form a part of soil structures (e.g. micro- and macro-aggregates) or be the principal component itself. Water repellency (WDPT > 5 s), caused by micro-aggregates, can already occur in wettable soils, in the finest (< 53 μm) out of ten sieve fractions. Water repellency of the finest sieve fraction did not affect the wettability of the whole sample because the other nine sieve fractions (53 μm to > 2000 μm) were wettable.

With increasing water repellency, from slightly to extremely water-repellent soils, more sieve fractions are occupied by soil structures and components that contain water-repellent organic remains, e.g. macro-aggregates, plant fragments and coatings on sand grains. Most of these structures and micro-aggregates have presumably, partly or wholly, been made by soil biota. Conversely, the degree of water repellency of soils can be reduced by decomposition of organic matter and by coating of plant fragments with fine non-water-repellent soil materials; processes in which soil biota are also active.

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INTRODUCTION

Many surface layers of sandy, clayey and peaty Dutch soils become, when dry, slightly to extremely water-repellent (Dekker and Bisdom, 1992). This study indicated that sandy soils in the Netherlands with heath, grass and forest covers are extremely water repellent, whereas surface layers of sandy soils under arable conditions are frequently wettable to slightly water-repellent. Water repellency affects the way in which rainwater penetrates the soil, thereby inducing preferential flow paths. As a consequence of preferential flow, water and solutes can reach the water-table more rapidly than in the case of a homogeneous infiltration front. This has consequences for the quality of the groundwater. A research project on occurrence and origin of water repellency, with the effects on preferential flow, is conducted at the DLO Winand Staring Centre.

This paper gives the results of a reconnaissance study on the relation between water repellency and soil constituents in sandy soils from a dune area near the village of Ouddorp in the western part of the Netherlands. No systematic study has been made with respect to forms of land use (grassland, arable land and uncultivated soils). Examples of the relation between water repellency and soil constituents are from 21 samples in 4 WDPT (water drop penetration time) classes. To this end the WDPTs of these samples and their sieve fractions (10 for each sample) were measured. Soil constituents of samples and sieve fractions, including soil structures presumably formed by soil biota, were examined using light and electron microscopes.

MATERIALS AND METHODS

Soils and climate

Sandy soils, mesic Typic Psammaquents (De Bakker, 1979), were studied. The soils have developed in dune sands near Ouddorp on the Island of Goeree, the Netherlands. Our examples are from A and C horizons above 50 cm depth. Clay, often rich in carbonate, was added in a number of locations in the past to improve soil fertility. The climate is of the Cfb-type according to Köppen's classification. Mean annual precipitation amounts to 765 mm and is evenly distributed. Potential evaporation averages 690 mm/yr. Mean monthly temperatures vary between 1.7°C in January and 17.0°C in July.

Water drop penetration time (WDPT)

The water repellency of a sample is measured with the water drop penetration time (WDPT) test, which is done by placing 3 water drops on an air-dry sample with a medicine dropper. The time needed to penetrate the sample is

recorded (Krammes and DeBano, 1965; DeBano, 1981). In this study we chose the median value of the three drops for Tables 2 and 3. A soil is considered water-repellent when the WDPT exceeds 5 s. The WDPT may increase to over 3600 s in an extremely water-repellent soil (Dekker and Jungerius, 1990).

WDPT tests were done on 21 samples which could thus be grouped in WDPT classes. The samples were dried at 60°C and left for at least two days outside the stove to equilibrate with the ambient air humidity in the laboratory. The dry samples were subsequently sieved into 10 dry fractions (< 53 μm , 53–106 μm , 106–150 μm , 150–212 μm , 212–300 μm , 300–425 μm , 425–500 μm , 500–1400 μm , 1400–2000 μm and > 2000 μm), washed on a sieve with mesh 53 μm (removal of fine and floating organic materials) and treated with hydrogen peroxide (removal of organic matter). Each of these treated samples was also subjected to the WDPT test.

Microscopy

At the start of the project 11 small thin sections (4.8 cm $\times$ 2.7 cm) were prepared using polyester resin (Jongerius and Heintzberger, 1975) and gamma radiation (Bisdorn et al., 1983) for hardening. These thin sections allowed an in situ light microscopic reconnaissance study of constituents in our sandy soils and a comparison of these with soil components mentioned in literature. Thereafter only unhardened samples from the same soils were used for light and electron microscopy (Bisdorn and Dekker, 1992). These 21 unhardened samples were derived from different WDPT classes.

RESULTS

Water repellency and soil constituents of WDPT classes

The WDPT test indicated that four of the five WDPT classes were represented in the 21 studied samples (Table 1). Nine samples were wettable and

TABLE 1

Classification of water repellency and distribution of 21 samples over 5 classes

WDPT (s)	Classification	Number of samples
< 5	Wettable	9
5–60	Slightly water repellent	3
60–600	Strongly water repellent	–
600–3600	Severely water repellent	3
> 3600	Extremely water repellent	6

the others slightly, severely or extremely water-repellent. Information on the distribution of wettable and water-repellent sieve fractions in the samples and on the constituents that caused the water-repellency of these samples was obtained from WDPT tests and microscopy of sieve fractions (Bisdome and Dekker, 1992).

WDPT class < 5 s

Five of the nine samples of wettable soils (Table 2) had a WDPT shorter than 5 s in all ten sieve fractions. The finest sieve fractions (< 53 µm) of samples 7 to 9 from wettable soils were strongly to severely water-repellent, with a maximum of 1337 s. Microscopy revealed that micro-aggregates, composed of fine organic materials and minerals, caused these high WDPT values.

Both macro-aggregates and micro-aggregates were often represented in the samples of our Typic Psammaquents. Macro-aggregates (Fig. 1) occupied sieve fractions larger than 300 µm and micro-aggregates (Fig. 2) sieve fractions smaller than 150 µm. The two intermediate sieve fractions (150–212 µm and 212–300 µm) acted as a barrier between the two types of aggregates, because macro-aggregates and micro-aggregates were usually not represented in these sieve fractions.

Macro-aggregates were composed of fine and coarser soil materials with and without plant remains. Macro-aggregates were important to the development of water repellency in severely and extremely water-repellent (600–3600 s) soils but played no role in wettable and slightly water-repellent soils.

TABLE 2
Water repellency (s) of sieve fractions from 9 samples in WDPT class < 5 s and 3 samples in WDPT class 5–60 s

Sieve fraction		Wettable								Slightly water repellent			
(No.) (µm)													
I	< 53	2	2	2	2	3	4	91	268	1337	30	180	2800
II	53–106	<1	<1	<1	<1	<1	<1	3	1	<1	3	22	15
III	106–150	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	7	<1
IV	150–212	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	2	<1
V	212–300	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	<1
VI	300–425	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	2	1
VII	425–500	<1	<1	<1	<1	<1	<1	<1	<1	<1	2	3	10
VIII	500–1400	<1	<1	<1	–	1	10	<1	–	<1	12	6	–
IX	1400–2000	<1	<1	<1	–	1	3	<1	–	<1	11	6	415
X	> 2000	<1	<1	<1	–	<1	2	<1	–	<1	<1	2	70
Sample No.		1	2	3	4	5	6	7	8	9	10	11	12

–: No measurement; too little or no soil material present in sieve fraction.

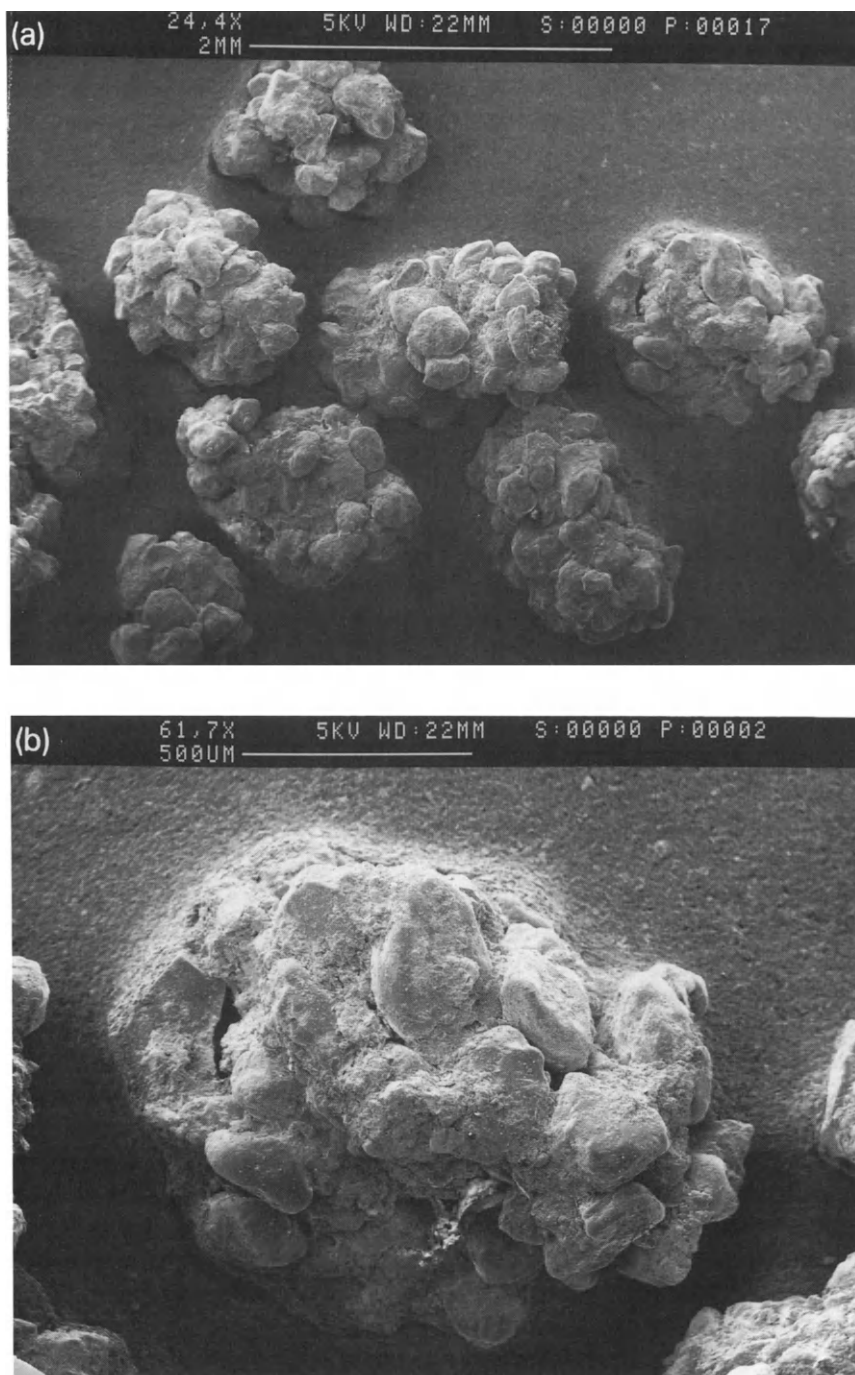


Fig. 1. (a) Rounded macro-aggregates, dominant in the sieve fraction 500–1400 μm of a wettable soil (WDPT class < 5 s). (b) Enlargement of the top right-hand macro-aggregate.

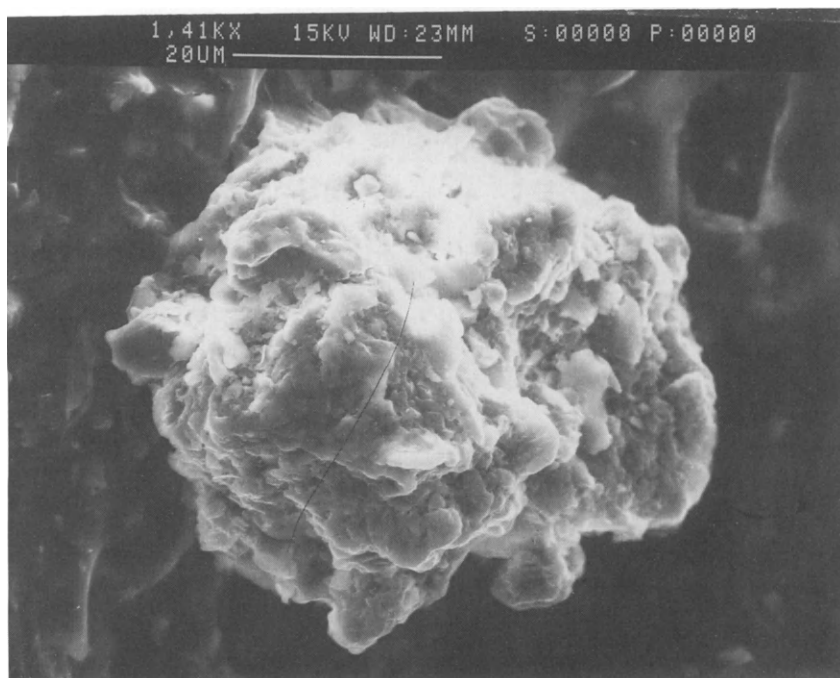


Fig. 2. Micro-aggregate, dominant in sieve fraction $< 53 \mu\text{m}$ of all soil samples.

WDPT class 5–60 s

In the slightly water-repellent WDPT class more sieve fractions (Table 2) and more types of soil constituents were responsible for the higher WDPT value of the samples than in the former WDPT class ($< 5 \text{ s}$). The two finest sieve fractions (I, II) of samples 11 and 12 were slightly to severely water-repellent. Strongly water-repellent conditions, with a maximum of 415 s, were found in the coarsest sieve fractions (IX, X) of sample 12. Micro-aggregates were still dominant in the fine sieve fractions according to microscopic observations. New were plant fragments, with and without a coating of fine soil materials (Fig. 3). These were dominant in coarser sieve fractions with a higher WDPT.

WDPT class 600–3600 s

Seven sieve fractions of sample 13 (Table 3) in the severely water-repellent WDPT class were slightly to severely water-repellent, whereas sieve fractions III, V and VI were wettable. All the sieve fractions of sample 14, with the exception of sieve fraction I (WDPT $> 3600 \text{ s}$), were severely water-repellent. Most sieve fractions of the severely water-repellent sample 15 were extremely water-repellent.

Compared with slightly water-repellent soils (Table 2), microscopy indi-

cated that more types of soil constituents in more sieve fractions caused the increase in WDPT of severely water-repellent soils (Bisdorf and Dekker, 1992). Micro-aggregates (Fig. 2) still dominated the finest sieve fractions (I–II). In the coarsest sieve fractions (VII–X) macro-aggregates, composed of fine soil materials with plant remains, were added to plant fragments with and without coatings (Fig. 3). The water repellency of four intermediate sieve fractions (III–VI) was mainly increased by thin speckles and dots (Fig. 4) of fine organic, mineral and amorphous materials. Speckles covered larger areas of the sand grains than dots.

WDPT class > 3600 s

In extremely water-repellent soils most of the sieve fractions have WDPT values of more than 3600 s (Table 3). Only in sample 16 two sieve fractions (III, V) were severely water repellent. Microscopy revealed that all the constituents of the former WDPT class, often in larger quantity, contributed to the extreme water repellency of these samples. New were platy fragments, found between sand grains and composed of fine organic matter, minerals and amorphous materials. These were predominantly represented in finer sieve fractions ($< 212 \mu\text{m}$).

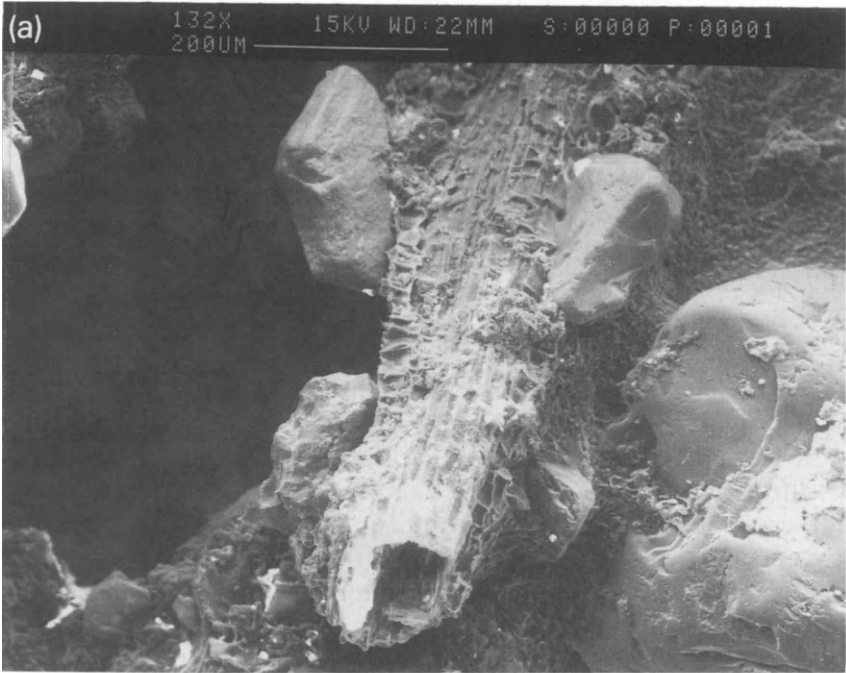
An exception formed sample 21 with an extreme water repellency in sieve fractions I to VII. In this case the water drops remained even more than six hours on the surface of the sample. This repellency was not caused by a combination of different soil constituents but, predominantly, by coatings only (Fig. 5).

Causes of water repellency

So far a number of soil constituents (micro-aggregates, plant fragments with and without coatings, macro-aggregates, speckles, dots, coatings and platy fragments) have been found in water-repellent sieve fractions. In this section our results on causes of water repellency in sandy soils are compared with causes mentioned in the literature, especially data on coatings and organic matter.

Coatings on and interstitial soil material between sand grains

In literature coatings are mentioned as one of the principal causes of water repellency in soils (Krammes and DeBano, 1965; Roberts and Carbon, 1972; King, 1981). Usually such coatings also contain organic matter (Bond, 1969; Miller and Wilkinson, 1977; Ma'shum et al., 1989). Coatings on sand grains, without the help of other soil constituents, caused an extreme water repellency in only one of our samples (No. 21, Table 3). The thin coatings consisted predominantly of amorphous substances. However, in our sandy soils,



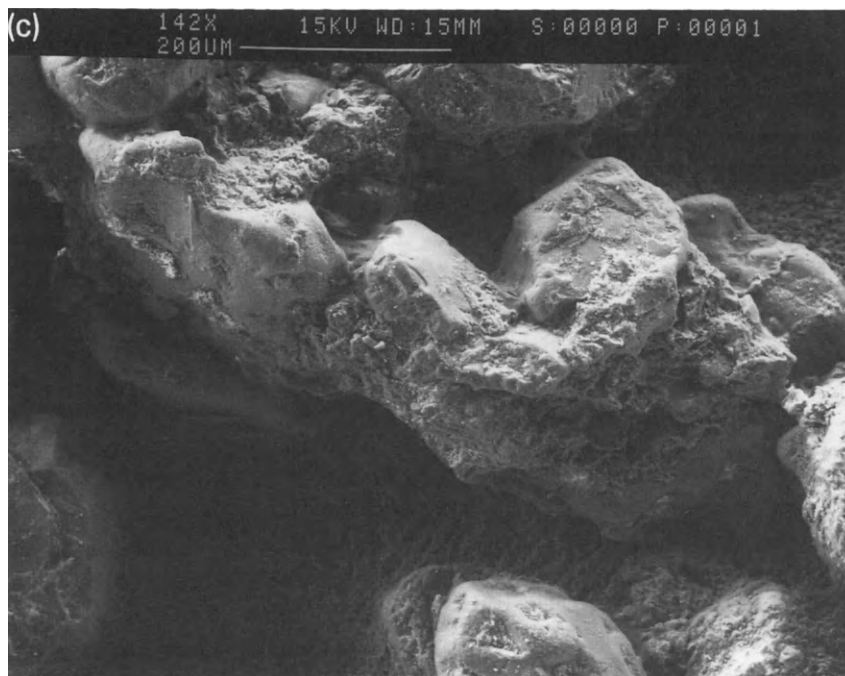


Fig. 3. Encrustation of plant fragments, different stages. (a) Plant remain partly coated by finer soil materials. (b) More strongly coated plant fragment; the fragment is still visible. (c) Virtually completely coated plant fragment.

mainly combinations of soil components caused water repellency of soil samples.

During the initial part of our study 11 thin sections were prepared to obtain information on coatings and on the nature, distribution, size, form, quantity, etc. of soil constituents and features. Coatings on sand grains (Fig. 5) were rare in our samples but fine soil material, with a comparable composition, occurred in larger quantities in pores between the sand grains. Consequently, our attention was drawn to these interstitial soil materials instead of coatings as a probable cause of water repellency. This interstitial material comprised mainly micro-aggregates, clay, fine silt, fine plant remains and amorphous substances of various composition.

To establish whether interstitial soil constituents between sand grains were indeed important to water repellency, all 21 samples were washed with water on a sieve. Floating (predominantly plant fragments) and fine, heavier (clay and finer quartz grains) soil materials were removed leaving mainly sand grains in the sieve. The WDPT decreased significantly in all cases but one. Speckles and dots (Fig. 4), of which a smaller number was still present on the surface of sand grains after washing, were the main cause of the remaining

TABLE 3

Water repellency (s) of sieve fractions from 3 samples in WDPT class 600-3600 s and 6 samples in WDPT class > 3600 s

Sieve fraction		Severely water repellent			Extremely water repellent					
(No.)	(μm)									
I	< 53	3208	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600
II	53–106	289	2301	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600
III	106–150	4	1808	> 3600	1878	> 3600	> 3600	> 3600	> 3600	> 3600
IV	150–212	7	2580	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600
V	212–300	1	2000	> 3600	2003	> 3600	> 3600	> 3600	> 3600	> 3600
VI	300–425	1	942	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600
VII	425–500	68	1998	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600	> 3600
VIII	500–1400	178	2745	–	> 3600	> 3600	> 3600	> 3600	> 3600	–
IX	1400–2000	512	3000	2980	> 3600	> 3600	> 3600	> 3600	> 3600	–
X	> 2000	347	1990	2530	> 3600	> 3600	> 3600	> 3600	> 3600	–
Sample No.		13	14	15	16	17	18	19	20	21

–: No measurement: too little or no soil material present in sieve fraction.

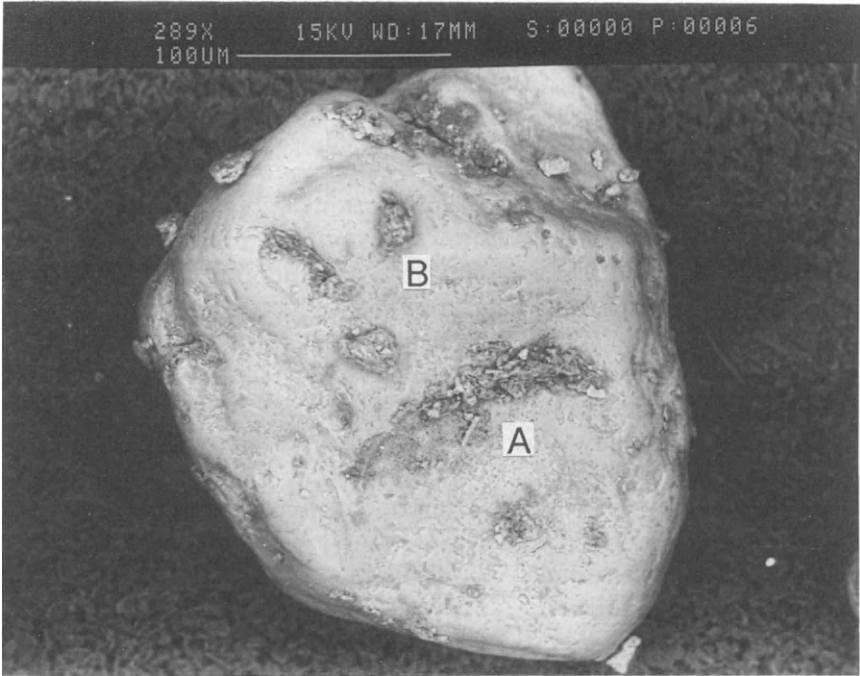


Fig. 4. Speckles (A) and dots (B) of fine soil material on the surface of a sand grain.



Fig. 5. Coating with fine soil material of a part of the surface of a sand grain.

water repellency of samples. The exception was sample No. 21 with coatings on sand grains and virtually no interstitial soil constituents to wash away. The coatings, which still covered a large part of the surface of sand grains after washing, kept the sample extremely water-repellent.

Organic matter

It is the organic matter which induces water repellency in soils by several means according to the review by DeBano (1981), namely by irreversible drying of organic matter, heating by fire of organic material, by organic substances leached from plant litter, by hydrophobic microbial by-products which coat mineral soil particles, and by intermixing of mineral soil particles with organic matter. A number of plant species can cause water repellency in soils, namely fungi, perennial pastures, citrus trees, various chaparral brush species, shrubs and trees (McGhie and Posner, 1981). A host of organic substances of unknown composition seems to be involved in water repellency (DeBano, 1981). The chemical characterization of substances which cause water repellency are often related to humic and fulvic acids.

In our study of sandy soils, combinations of soil constituents and coatings with organic matter were found to cause water repellency. The plant remains involved in water repellency were remnants of roots, leaves and stems. Dur-

ing the research project on occurrence and origin of water repellency in the Ouddorp area, over 4000 samples were subjected to the WDPT test and it was found that most organic materials are potentially water-repellent (Dekker and Bisdom, 1992).

To prove that organic substances were indeed responsible for the phenomenon of water repellency, the 21 samples were treated with hydrogen peroxide after which all samples became wettable (WDPT < 5 s). Microscopy revealed that most speckles, dots and coatings (Figs. 4 and 5) were removed from the surface of sand grains. Plant remains and soil structures were destroyed. Predominantly clean, white sand grains remained. These results confirm that organic matter is the main cause of water repellency.

Reduction of water repellency

Decomposition of organic material

Processes that destroy organic substances with respect to water repellency, are important. Biological decomposition of leaves, stems and roots, whereby different types of humus and organic substances are formed, will give different WDPT values (Dekker and Bisdom, 1992). In general, fresh and partly decomposed organic matter will have a higher WDPT than more completely humified fragments. During this biodegradation of the biomass organic macromolecules are broken down and water repellency will be reduced.

Deactivation of organic material

Deactivation of soil organic matter with respect to water repellency can also be important to the reduction of water repellency (Bisdom and Dekker, 1992). This bioprocess takes place when water-repellent plant fragments (roots, stems, leaves) are partly or wholly coated (Fig. 3) with wettable (fine) soil materials (clay, quartz, amorphous substances). If organic matter is present in the coated plant fragments, wettable to extremely water-repellent conditions are possible. Addition of calcareous clay for soil improvement by farmers reduced water repellency often considerably. Coating of plant fragments with this wettable material played an important role in this reduction. These soils, improved with clay, are commonly wettable to slightly water-repellent.

Coating of roots, and presumably of leave and stem fragments, is a complex process. Organic exudates of roots, microorganisms and minerals combine in the rhizosphere to form coatings (Tisdall and Oades, 1979; Foster et al., 1983; Oades and Waters, 1991). Soil biota are therefore important to the development of coated plant fragment structures as found in our sieve fractions.

Macro- and micro-aggregates are other soil structures that can partly or wholly enclose plant fragments with soil material (clay, quartz, amorphous substances, organic remnants) and thereby reduce their water repellency. De-

pending on the degree of water repellency of the enclosing soil material the macro- and micro-aggregates can themselves be slightly to extremely water-repellent or wettable. Examples are rounded macro-aggregates (Fig. 1) and a rounded micro-aggregate (Fig. 2).

Rounded macro-aggregates were well developed in the sieve fraction 500–1400 μm (VIII) and rounded micro-aggregates in sieve fractions $< 106 \mu\text{m}$ (I, II). The rounded macro-aggregates occurred in wettable samples from soils to which farmers added calcareous clay for soil improvement. Rounded micro-aggregates can occur in wettable and slightly to extremely water-repellent soils (Bisdorf and Dekker, 1992).

Both types of aggregates are rather similar in appearance when the micro-aggregates (Fig. 2) are sufficiently enlarged to compare with the macro-aggregates (Fig. 1). These rounded macro- and micro-aggregate structures were presumably mainly formed by bioprocesses and are interpreted as faecal pellets or excrements made by soil biota.

CONCLUSIONS

The appearance and degree of water repellency are closely related with organic matter. Plant remains, with and without coatings (Fig. 3), can be found in a number of soil constituents, viz. macro- and micro-aggregates (Figs. 1 and 2), speckles-dots-coatings (Figs. 4 and 5) on sand grains, and platy fragments between sand grains. These soil components and soil structures are differently distributed over the wettable to extremely water-repellent sandy soils. Micro-aggregates can initiate water repellency (max. 1337 s) in the finest sieve fraction of still wettable (< 5 s) soils, whereas combinations of soil constituents often induced the higher WDPT values of water repellent soils. The use of hydrogen peroxide dissolved organic matter and reduced the WDPT to zero.

The study of soil structures and organic matter, only in thin sections, has its limitations. Microscopy of sieve fractions gives valuable additional information on soil structures and organic matter. No WDPT measurements can be done on thin sections and soil particles cannot be moved for microscopic observation from all directions. The use of sieve fractions and their WDPT values allows to determine which individual or combination of soil constituents causes the water repellency of sieve fractions, samples and soils. Among these constituents were soil structures, namely micro-aggregates, rounded macro-aggregates and coated plant remains presumably formed by soil biota.

Experiments are necessary to establish the role of soil biota in the formation of soil structures and in the decomposition of organic matter in relation to water repellency. These should allow a step by step approach by which the field and experimental (micro-)structures of sandy soils can be compared.

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The physical structure of casts of *Millsonia anomala* (Oligochaeta: Megascolecidae) in shrub savanna soils (Côte d'Ivoire)

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ABSTRACT

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A tropical geophagous earthworm species, *Millsonia anomala*, is mainly responsible for the formation and the conservation of the structure of the upper horizons of soil (Ferralsol) at Lamto (Côte d'Ivoire). Its action on soil organic matter dynamics is important as organic matter in their water-stable casts is protected against mineralization.

This study aims at describing the physical structure of these casts in order to explain the protection of organic matter through the cast structure and to emphasize the role of earthworms on the soil structure.

The pore size distribution (mercury porosimetry) and the bulk density of casts were measured and casts were studied using thin sections and scanning electron microscopy observations. Casts collected at the surface of a shrub savanna soil and from earthworm cultures were analysed.

The results show that casts of *M. anomala* are very dense (bulk density ranges from 1.8 to 2.0 g cm⁻³) and that they have an important volume of pores with a diameter of ca. 10 µm. On the outside, these casts display a dense layer of ca. 25 µm thick. This cortex consists of fine particles and can be expected to impede water and air movements. This particular physical structure may explain the protection of organic matter in casts, that has been observed by other authors.

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INTRODUCTION

To sustain the fertility of tropical soils, more research is needed on the effects of soil macroorganisms on soil structure and organic matter dynamics (Swift and Lavelle, 1987; Anderson and Flanagan, 1989; Lavelle et al., 1992c).

In moist African savannas, earthworms are often the dominant group of soil macrofauna and their influence on soil structure and organic matter dynamics has often been demonstrated (Lal, 1987, 1988; Lavelle, 1988; Lavelle et al., 1989, 1991b). At Lamto (Côte d'Ivoire), earthworms ingest large amounts of soil that they egest as casts which form round macroaggregates (5 to 20 mm diameter). In this way they are responsible for the formation and conservation of the macroaggregate structure observed in the upper 15 cm of soil (Blanchart, 1992; Blanchart et al., 1989, 1990). *Millsonia anomala* is the dominant species and with its 200 kg live weight per ha it may annually ingest ca. 480 Mg dry soil per ha in a shrub savanna, as measured in 1972 (Lavelle, 1978).

The role of *M. anomala* on dynamics of organic matter has been studied by Martin and Lavelle (1992) and Lavelle et al. (1992a). The ingestion of soil by *M. anomala* strongly influences the dynamics of soil organic matter both in the short and long terms. At a short time scale, digestion leads to an increased mineralization (2 to 6% of organic matter are assimilated during the gut transit) (Lavelle, 1978) while at a longer time scale, mineralization is blocked and organic matter seems to be protected in casts (Martin, 1991). Martin (1991) found that the C content in the control soil decreased more rapidly than in casts and that the mineralization rate was three times higher in the control as compared to casts, $11.1\% \text{ yr}^{-1}$ and $3.4\% \text{ yr}^{-1}$, respectively. This inhibition of decomposition may be due to both chemical (high amount of resistant humic compounds; Toutain, 1987) or physical factors (formation of stable clay-organic complexes; Greenland, 1965; Marshman and Marshall, 1981 in McKeague et al., 1986; Emerson et al., 1986). The soil micro- and macro-structure may also play a role: it is possible that organic matter is not accessible to microorganisms when it is located in very small pores whose diameter is smaller than the diameter of microorganisms (fungal hyphae are 1 to 20 μm in diameter and other microorganisms are 0.3 to more than 50 μm in diameter). A macro-structure which does not allow sufficient aeration or water infiltration may create anaerobic conditions (Adu and Oades, 1978; Elliot et al., 1990) that also slows down the decomposition.

The aim of this study was to describe the physical structure of the casts of *M. anomala* to test whether the protection of organic matter may result from a physical process and to emphasize the role of *M. anomala* on the structure of the soil of Lamto's savanna. The total porosity and the pore size distribution of casts of *M. anomala* were studied using a mercury intrusion porosimeter. The bulk density of casts was measured and their physical structure

described using thin sections and SEM (scanning electron microscopy) observations. Casts collected from cultures of worms and casts collected at the surface of a shrub savanna soil were described.

MATERIALS AND METHODS

Study site

This study was carried out at the Station d'Ecologie Tropicale de Lamto in Côte d'Ivoire (5°02'W, 6°13'N, elevation 105 m). The soils are infertile tropical ferruginous soils (Ferralsols, FAO-Unesco classification) derived from granitic parent materials. The upper 10 cm in shrub savannas are characterized by high sand and low clay contents, ca. 75% and ca. 7.5% respectively. They have low organic matter (ca. 1% carbon) and exchangeable cation contents (3.2 cmol p(+) kg⁻¹) (Blanchart, 1990). In 1987, the mean annual temperature was 28.8°C and annual rainfall reached 1100 mm. The rainy season occurs from April to July and from September to November.

In shrub savannas, the earthworm community is dominated by *Millsonia anomala*, a medium-sized, endogeic, mesohumic geophagous species (mean biomass 21.1 g live weight m⁻², i.e., 50% of the total earthworm biomass as measured in 1972; Lavelle, 1978).

Assessment of pore size distribution by mercury porosimetry

Description of the method

This method involves both measurement of the pressure required to force mercury into the pores of a dry sample and of the volume V_m of intruded mercury at each pressure. Both the pressure and the volume were measured using a porosimeter (Micromeritics 9310) (characteristics described by Fiès and Bruand, 1990). An inverse relationship exists between the applied pressure P and the equivalent pore diameter D (Lawrence, 1977; Fiès and Bruand, 1990). The pressure varied between 3×10^{-3} MPa and 200 MPa. The corresponding D values ranged from 5.6×10^2 to 6.2×10^{-3} μm . The cumulative pore volume (cpv) curve was constructed starting from the smallest pores. It is used to establish the pore volume distribution (pvd) curve, which is the derivative curve. Each point represents the slope of the cpv curve between two successive values of D , calculated using the expression $\Delta V_m / \Delta \log D$ (Coulon and Bruand, 1989).

Preparation of the samples

Five types of cast derived from both laboratory cultures and the field were analysed. For the laboratory cultures, some young earthworms were collected in the upper centimeters of a shrub savanna soil. The breeding soil came from

the upper 10 cm of a shrub savanna; it was air-dried and passed through a 2 mm sieve to extract coarse roots. Then it was moistened ($pF=2.5$, i.e. 12% humidity), passed through a 2 mm sieve, placed at 28°C in growth chambers and earthworms were added. After 2 days, casts were hand-sorted. In this way, two types of casts were separated, namely casts collected from a culture of: (1) earthworms with a fresh weight of ca. 0.5 g (code name: EW0.5), (2) earthworms with a fresh weight of ca. 1.8 g (EW1.8).

Furthermore, artificial casts were made using casts that had been mixed with water and then dried. This sample is called cast-paste (PAST). Two type of casts were taken from the field: a cast collected at the soil surface in a shrub savanna (SURF) and a cast collected at the soil surface in a shrub savanna and which has been fractured (a small part has been cut off) (FRAC).

Bulk density of casts

The bulk density of casts was measured using the kerosene impregnation method (Monnier et al., 1973). The measurements were obtained from 7 casts collected from the culture of earthworms and 3 casts collected from the soil surface in a shrub savanna.

Thin sections

Thin sections were obtained from soil monoliths collected from the upper 12 cm of a shrub savanna. The samples were impregnated with a polyester resin. Addition of a fluorescent dye to the impregnating mixture enabled pores to be seen in the thin sections under UV light (Fitzpatrick, 1970).

Scanning electron microscopy

Internal and external structures of air-dried casts collected from earthworm cultures (EW1.8) and at the soil surface in a shrub savanna (FRAC) were observed using a scanning electron microscope (JEOL JSM 35) at the University of Rennes (France).

RESULTS AND INTERPRETATION

Pore size distribution

The total volume of mercury intruded (V_m) and thus the total volume of pores was similar for the four types of casts (ca. $0.17\text{ cm}^3\text{ g}^{-1}$) and higher for PAST (cast-paste) ($0.22\text{ cm}^3\text{ g}^{-1}$) (Table 1). The pore space is essentially constituted of pores which range from 5.6 to $29\text{ }\mu\text{m}$ diameter except for PAST that also has pores of $10^2\text{ }\mu\text{m}$ (pvd curves, Fig. 1).

TABLE 1

Total porosity, bulk volume and bulk density of different types of casts of *Millsonia anomala* (legend in text)

	EW0.5	EW1.8	SURF	FRAC	PAST
Volume of mercury intruded, V_m (cm ³ g ⁻¹)	0.1672	0.1722	0.1714	0.1782	0.2193
Bulk volume (kerosen method) (cm ³ g ⁻¹)	–	0.508 ±0.016	0.555 ±0.015	–	–
Bulk density (g cm ⁻³)	–	1.97	1.80	–	–

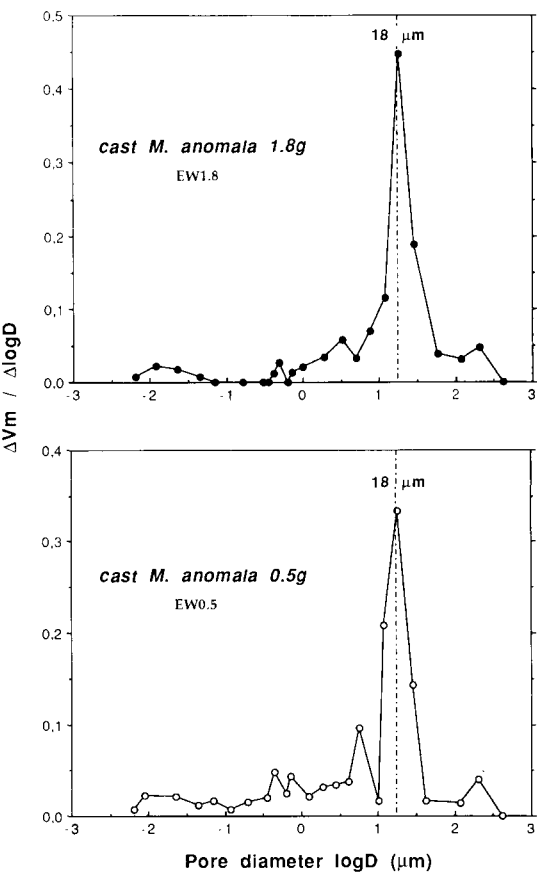


Fig. 1. Pore volume distribution (pvd) curves for different types of casts of *Millsonia anomala* (explanation in text). V_m =volume of mercury intruded into the samples. Note that the scale of the y-axis differs for the different types of casts.

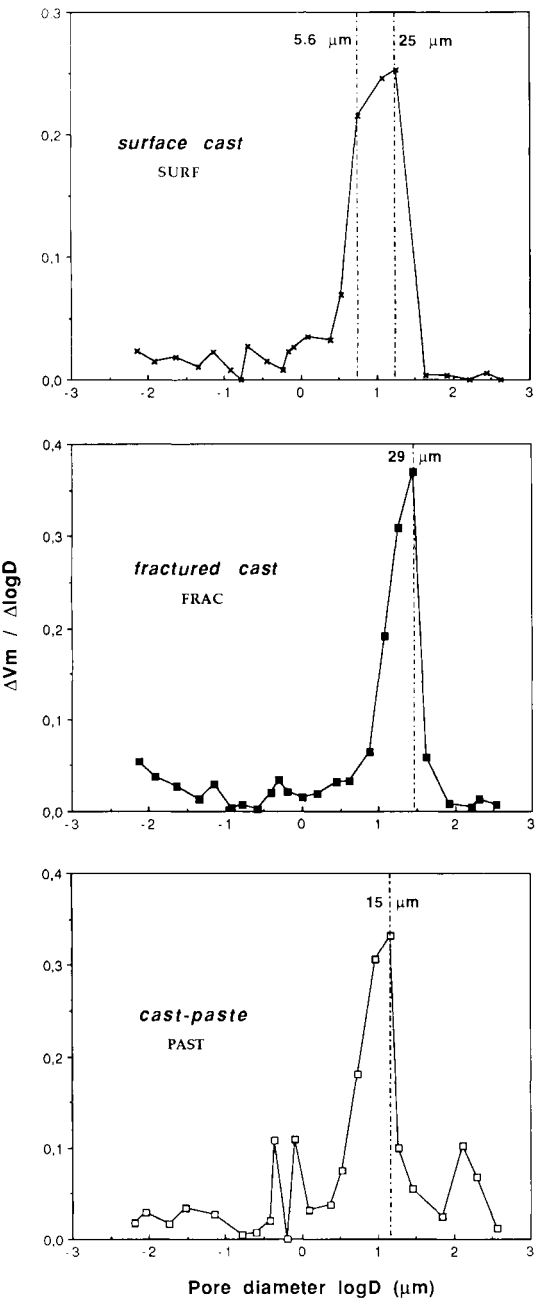


Fig. 1. Continued.

The pvd curves show that the intrusion peak of mercury for SURF is between pressures that have the equivalent pore sizes: 5.6 to 25 μm (Fig. 1). PAST, EW0.5, EW1.8, and FRAC curves present a narrow peak for pores which have an equivalent diameter of 15, 18, 18, and 29 μm diameter, respectively. SURF presents a very weak intrusion of mercury for pores wider than 25 μm diameter.

Bulk density of casts

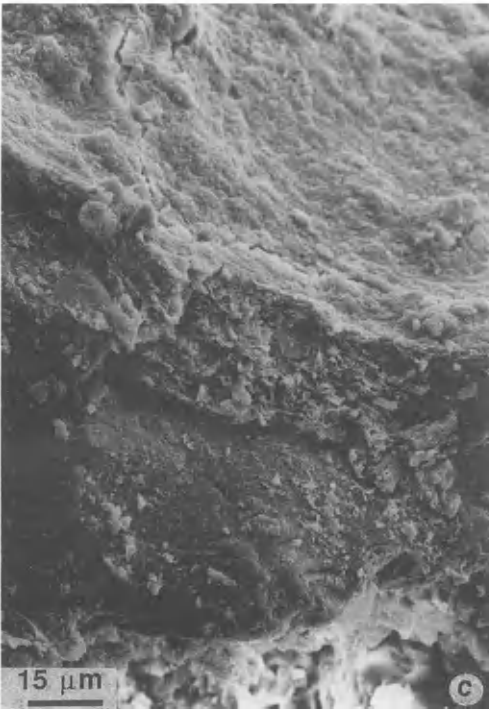
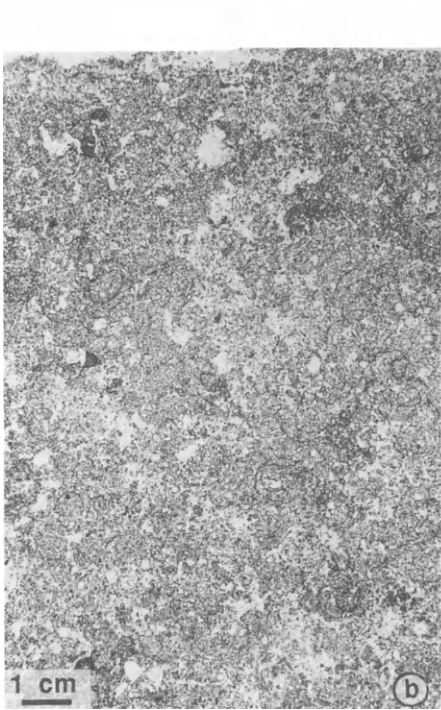
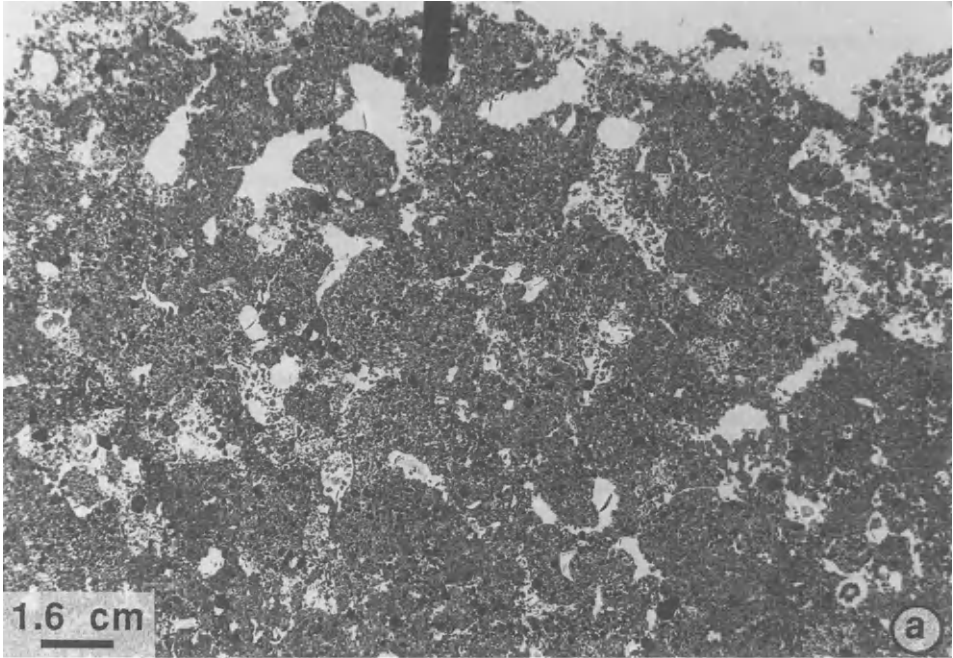
The casts collected from the culture of worms (EW1.8) have a mean bulk volume equal to 0.508 $\text{cm}^3 \text{g}^{-1}$ (standard deviation 0.016) which corresponds to a high bulk density of 1.97 g cm^{-3} (total porosity: 25.1%) (Table 1). The casts collected from the soil surface (SURF) have a mean bulk volume of 0.555 $\text{cm}^3 \text{g}^{-1}$ (standard deviation 0.015), i.e., a bulk density equal to 1.80 g cm^{-3} (total porosity 31.6%).

Soil thin section and SEM observations

On a thin section observed under UV light, the casts are easily recognizable in the section because of their high density (Fig. 2a). Almost the whole section consists of casts except for a few macropores and some less compacted areas. Such a soil is characteristic for the activities of medium-sized worms like *M. anomala* (Blanchart, 1992). Using normal light (Fig. 2b) the outlines of the casts stand out clearly. The peripheral layers of the casts of *M. anomala* are compact and composed of fine organic and inorganic particles while the inner parts are composed of a mixture of coarse and fine particles. The difference between the two parts can be observed in the fractured cast with a scanning electron microscope (Fig. 2c). The cortex is ca. 25 μm thick and gives the surface casts a smooth aspect. The surface of a cast collected from the culture of worms is different from that of a soil surface cast (Fig. 2d). The cultured cast displays a rough surface because the cortex only appears in some places. Silt and sand particles are thus visible at the cast surface. The cortex may have been destroyed when sampling the casts.

Interpretation of pore size distribution results

The very weak mercury intrusion observed in the surface cast (SURF) for pores wider than 25 μm may be explained by the presence of the cortex which prevents mercury intrusion at low pressures and, hence, internal wide pores are inaccessible to mercury. From a pressure of 41.3×10^{-3} MPa (equivalent pore diameter 25 μm), mercury begins to intrude and fills the internal pores wider than 25 μm . This explains the high volume of mercury intruded under pressures ranging from 41.3×10^{-3} to 220.5×10^{-3} MPa ($D=5.6 \mu\text{m}$). The



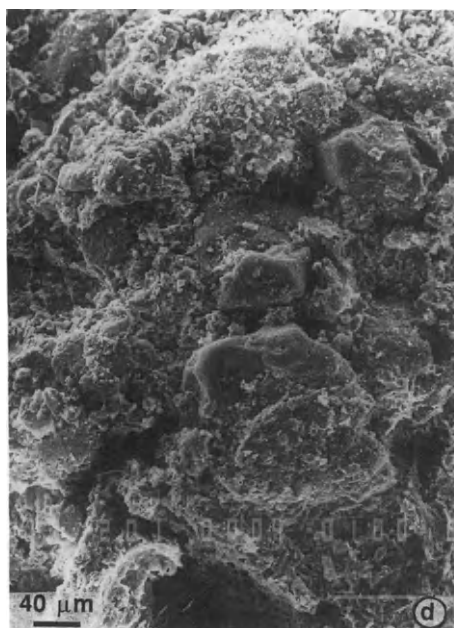


Fig. 2. Thin soil sections and scanning electron microscopy (SEM) photographs. (a) Thin section of the upper 5 cm of soil collected in a shrub savanna at Lamto, observed under UV light. Voids are white. Observe the compaction in casts essentially due to *Millsonia anomala*. (b) Thin section of the upper 9 cm of soil collected in a shrub savanna at Lamto, observed under normal light. Observe the outlines of casts created by *M. anomala* (arrows). (c) Fracture of a surface cast of *M. anomala* observed with SEM. Observe the cortex made of fine particules that gives the surface of the cast a smooth and close aspect. (d) Surface of a cast of *M. anomala* collected from a culture of worms and observed with SEM. Note that the surface is rough and porous because of the absence of a cortex.

curve of the fractured cast (FRAC) confirms the importance of the cortex in fluid penetration as in this sample, mercury can moderately intrude under the low pressures only through the fractured part. For the casts collected in culture soil (EW0.5 and EW1.8), the mercury intrusion is moderate at low pressures. This can be explained by a lack of cortex. Finally, the cast-paste (PAST) was different from earthworm casts. The mercury intrusion was important at low pressures, thus proving the absence of a cortex in this sample, a higher porosity and/or larger pores.

DISCUSSION AND CONCLUSION

Porosity measurements indicate that the crust surface of casts, fully observed in SURF, fractured in FRAC and only partly present in EW0.5 and EW1.8

prevented connection between the internal pores and the outside of the casts. Reabsorption of water in the latter part of the gut is probably responsible for the formation of the cast-cortex as the fine particles (clays and fine organic particles) are drawn by water towards the periphery of the cast before they are stopped by the gut tissues. Then the casts are excreted. Casts collected from the soil surface in the field showed an intact cortex (Fig. 2c). The cast-cortex is conspicuous on a thin section surface of undisturbed field soil (normal light, Fig. 2b) while it is absent in casts from the laboratory (Fig. 2d). The reason may be that the cortex of casts egested inside the soil were partly removed when the casts were sampled from the soil.

Moreover, the bulk density of these sandy casts is very high. It is higher than the bulk density measured for casts of other endogeic earthworms such as *Aporrectodea rosea* (mean bulk density 1.15 g cm^{-3}) bred in a sandy loam soil (McKenzie and Dexter, 1987). The compact structure of the casts of *M. anomala* may result from three phenomena: (i) the formation of organo-mineral bonds after the intense mixing and chemical transformation associated with gut transit, (ii) the reabsorption of water in the latter part of the gut, and (iii) strong compaction due to the muscles of the tail region when casts are expelled (McKenzie and Dexter, 1987). Hence, the cast of *M. anomala* is not a simple assembly of particles obtained by mixing with water. This was confirmed by the differences in the porosity measurements between PAST and the other samples. The combination of surface crust and high bulk densities can be expected to favour anaerobic conditions which may cause retardation in the decomposition of organic materials in the casts (Elliot et al., 1990).

The large size of casts may also affect oxygen-diffusion into the cast; several authors have shown that mineralization of N, nitrification and O_2 uptake are more rapid in smaller aggregates, as aeration is better than in larger aggregates (review in Adu and Oades, 1978). Sextone et al. (1985, in Brady, 1990) showed that in a wet aggregate measuring 18 mm in diameter, the oxygen content near the aggregate centre was 0% while it was 21% near the edge of the aggregate. Furthermore, when casts are egested, their water-stability is low (Shipitalo and Protz, 1989; Blanchart, 1990; Marinissen and Dexter, 1990) and C mineralization, which began in the gut, continues for some weeks (Martin, 1991). Gradually, a closer relation between clay and organic matter may develop probably due to wetting-drying cycles and thixotropic changes (Utomo and Dexter, 1981; Moloje et al., 1985; Shipitalo and Protz, 1989). This may increase the stability of casts and inhibit the decomposition of organic matter.

Indeed, Martin (1991) found that at long time scales (months and years), soil organic matter is protected within compact earthworm casts and the C content of these casts became higher than that of a 2 mm sieved soil control after 2 months of incubation. The casts were collected by Martin (1991) in

the same way that we did. Thus, it seems obvious that the cortex of the casts she used was missing. Two conclusions can be drawn: (1) the cortex has a limited role in the retardation of decomposition and hence the high density and big size of casts could explain the physical protection of organic matter, (2) the cortex has an important role and thus the retardation of decomposition shown by Martin under laboratory conditions is more important in the field. We could arrive at the right conclusion after comparing the protection of organic matter in casts from culture soil and in surface casts which present a cortex.

Our results give some information about the structure of the soil of Lamto. Indeed, the high densities of casts (1.97 g cm^{-3}) is responsible for the high bulk density of the upper centimetres of soil: $1.52 \pm 0.04 \text{ g cm}^{-3}$ between 0 and 10 cm and $1.53 \pm 0.04 \text{ g cm}^{-3}$ between 10 and 20 cm as measured in June 1988 (rainy season) (Blanchart, 1990). Blanchart et al. (1990) found that 65% of the soil weight was constituted of casts. In a soil block of 100 g (volume = 65.8 cm^3), the casts weighing 65 g represent a volume equal to 33 cm^3 (50%), while the non-cast soil (soil not compacted in casts and macropores) weighs 35 g and represents a volume equal to 33 cm^3 too (Fig. 3). Following this approximation, the non-cast soil has a bulk density of ca. 1.07 g cm^{-3} . Greenland (1977, in Hamblin, 1985) divided pores into three size classes: transmission ($> 50 \mu\text{m}$), storage (0.5 to $50 \mu\text{m}$) and residual ($< 0.5 \mu\text{m}$) pores. Thus, in the savanna soil of Lamto, the storage and residual pores which allow limited water movements are mainly found in the casts while the transmission pores are located between the casts. Thus, when the soil is moist, the small pores of the cast, specially of the cortex, hold water with a greater tenac-

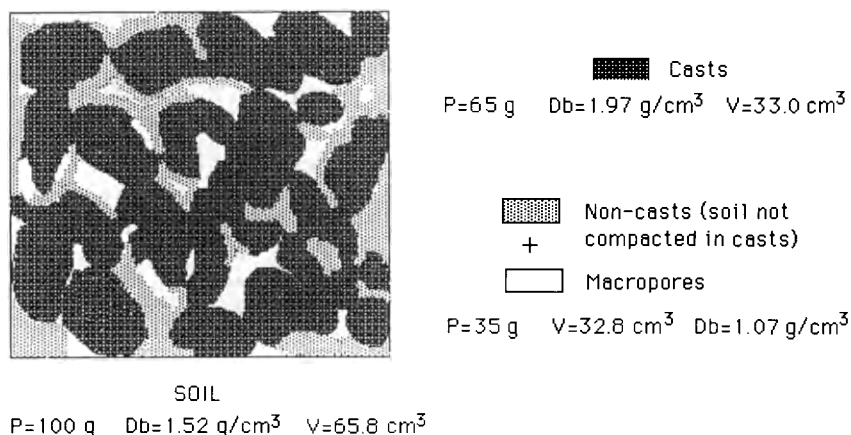


Fig. 3. Schematic representation of a soil block showing the casts, the macropores and the soil not compacted in casts. Because of their high bulk densities, the casts occupy half of the soil volume and two thirds of the soil weight. In the soil, the macropores are located between the casts while the storage pores are located in the casts. P =weight, V =volume, Db =bulk density.

ity than do larger pores. We can then infer that aeration is impeded in these aggregates and that the casts keep moist for a longer time than does non-compacted soil in the cast. *M. anomala*, by the excretion of casts, affects the soil air and water space, as well as gaseous exchange and biochemical reactions.

The casts can provide physical protection that can be expected to play a major role in the retardation of organic matter breakdown. However, changes in the chemical composition may also play a role and it would thus be necessary to study the different types of organic compounds (protein, polysaccharides, humic substances, etc.) present in the casts.

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Use of the minirhizotron–miniature video camera technique for measuring root dynamics

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ABSTRACT

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Root measurement techniques for annual row crops that are non-destructive, quick and efficient, and quantitative measures of root dynamics are critical to understanding environmental effects on rooting. This paper describes, utilizing data from Minirhizotron (MR) and miniature color video camera recordings, wheat (*Triticum aestivum* L.) and peanut (*Arachis hypogae* L.) seasonal rooting habits. Data for multiple and linear regression analysis were acquired by making root count (C_a , counts cm^{-2} MR) and root length (L_a , cm root cm^{-2} MR) measurements on the surface of MR during the plant growing season. Roots on the surface of MR were recorded on video cassettes, displayed on a video screen, and traced on cellulose acetate each sampling date. Root dynamics were determined by comparing successive tracings. Wheat root length density (L_v , cm root cm^{-3} soil) and root dry weight (W_v , g root cm^{-3} soil) were determined from soil cores. A correlation coefficient of 0.88 for a multiple regression expression describing C_a as a function of cultivar, sampling date, and soil depth was obtained. Linear regression for C_a as function of L_v , W_v , or L_a were highly significant, and correlation coefficients with C_a were 0.72, 0.69, and 0.82, respectively.

INTRODUCTION

Many destructive techniques are available for measuring root response to environment (Upchurch and Taylor, 1990), but these are usually laborious and are often not suited to evaluating root dynamics. The study of root dynamics using soil cores is subject to several problems (Singh et al., 1984). Observation tubes, using simple mirrors and a light source to study root growth, have given reasonable correlations between washed root lengths and in situ measurements of root length (Gregory, 1979; Bragg et al., 1983; Vos

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and Groenwold, 1987). Nondestructive measurements of root weight and dynamics are more efficient with the MR technique, which uses a miniature video camera (Upchurch and Ritchie, 1984). Correlations (r) for wheat (*Triticum aestivum* L.) of 0.93 for L_a and 0.95 for C_a with L_v determined from soil cores were reported for their video camera-MR system by Belford and Henderson (1985). But, they presented no data on root dynamics. Box and Johnson (1987) demonstrated seasonal variations in MR C_a that are associated with wheat plant phenological development, soil depth, and soil aeration. Vos and Groenwold (1987) presented MR observations that showed patterns of root change with time and depth similar to those commonly observed with the auger method. Their data were from container experiments for wheat and potato (*Solanum tuberosum* L.) and field experiments with potato and field bean (*Vicia faba* L.). Cheng et al. (1990, 1991) demonstrated that root production and turnover of grain sorghum [*Sorghum bicolor* (L.) Monech] can be studied in situ by combining the MR technique with the soil coring method and hand tracing of video tapes from MR. The purposes of these two experiments were to evaluate the use of MR data, utilizing multiple and linear regression, for describing seasonal changes of winter wheat L_v and W_v in relation to C_a and L_a ; and to evaluate a technique, utilizing L_a , for calculating peanut root turnover index (TI, %).

MATERIALS AND METHODS

Both the wheat and peanut studies were performed with recommended cultural practices in the field.

The equipment and techniques for installing the MR have been previously described (Box and Johnson, 1987; Box et al., 1989). A Giddings hydraulic power soil sample (Model GSR-T-S Giddings Machine Co., Fort Collins, CO), with a swinging mast and rotary head, was mounted on a 4230 John Deere tractor (John Deere, Moline, IL) at an acute angle of 1.2 rad for the wheat study and 1.05 rad for the peanut study from the soil surface (Bragg et al., 1983). A hole was bored into the soil to a depth of 1.20 m using a 0.04 m diameter spiral ship auger that was 0.90 m long and attached to a 2.40 m Kelly bar (25 mm square by 3 mm wall thickness steel tube). Soil holes were sized with a Giddings soil sampling tube that had a cutting point with an outside diameter of 55 mm and a cutting edge tapered towards the inside (Box et al., 1989, fig. 1). Minirhizotron tubes (51 mm inside diameter by 1.22 m length by 3.2 mm wall thickness polycarbonate) were scored on the outside top surface with a 1 mm wide etching pen along their lengths (transects) and were cross-etched at 0.10 m intervals. A plug for the bottom end of the tube was formed from a 38 mm length of 51 mm diameter solid polycarbonate rod. A 0.8 rad taper was cut on 25 mm of the plug and the remaining 13 mm was glued to the inside end of the tube with the taper forming a cone-shaped end.

Weld-on 4 solvent, (Industrial Polychemical Service, Gardena, CA) was used to glue the polycarbonate tube and plug together.

Minirhizotron tubes were pressed into the soil hole with the Kelly bar positioned inside the tube and against the plug at the bottom end. The effect of this installation procedure was to place a stretching force on the tube during installation into the soil hole. After the Kelly bar force was released the tube appeared to relax, expanding firmly against the soil. This procedure avoided the adhesion problem at the plastic–soil interface (Taylor and Böhm, 1976). The tube–soil contact was observed immediately following installation and each time root counts were made, and no problems with tube–soil contact were observed. The soil appeared to be firmly in contact with the tube surface. No soil voids were observed that appeared to affect root count along the tube–soil interface. Light exposed minirhizotron surfaces, which protruded 0.10 m above the soil, were made opaque by wrapping them with black plastic electrical tape. Protruding tube ends were closed with No. 11 neoprene stoppers. A light leak at any point is propagated via the tube walls throughout the tube interior. A neoprene house roof flange for a 50 mm diameter vent pipe was installed on the minirhizotron to assure a light seal at the soil surface–plastic tube junction. The flashing's flat portion was trimmed from the cup prior to installation. Light elimination from the stoppered minirhizotron tube was assessed with unexposed black and white photographic film (Levan et al., 1987).

Root counts (C_a) using a miniature video camera inserted into the minirhizotron tube were as described by Upchurch and Ritchie (1984). Specifically, each root and root branch were counted within each 0.10 m soil depth increment. Any root segment not visibly attached to another root was counted as a root.

Wheat study

Soft red winter wheat was grown on a Cecil sandy loam (clayey, kaolinitic, thermic Typic Kanhapludult) Watkinsville, Georgia. Root data from minirhizotrons and soil cores were compared statistically. The minirhizotron data were collected during the 1987–1988 cropping season. Treatment plots were 25.2 m wide and 30.3 m long. Tillage of the soil following summer fallow occurred between 1 October and 6 October 1987 and included chiseling to 0.2 m and disking, followed by drag harrowing. Seeding at the rate of 10.7 g m⁻² in drill rows 0.12 m apart was done on 29 October 1987.

Within a treatment plot, a soil core was taken randomly in each of the two drill rows that were occupied by MR. The soil core was located 2.5 m away from the end of the plot. Soil cores (41 mm in diameter) were taken to a depth of 1.0 m, cut into 0.15 m lengths, placed in sealed polyethylene bags, and stored at 5°C. Roots were prepared by washing them from the soil cores with a hydropneumatic elutriator (Smucker et al., 1982) and staining them

with a methyl violet solution (10^{-4} g in 1% ethyl alcohol). Root lengths were determined on a Delta-T analyzer (Decagon Devices, Pullman, WA) that uses a line-intersect method. Installation of MR was 10 days after planting. Eight MR were installed per plot in the four center rows. The MR were placed 1.5 and 2.5 m from a plot end and reclined parallel to the rows and toward the plot end.

The statistical design was a randomized block with three replications. Three soft red winter wheat cultivars, Hunter, Stacy, and Florida 302, were the treatments. Root count (C_a) and L_a from each 0.15 m depth increment of the eight MR in a treatment plot were each averaged. Root data from the two soil cores were averaged for each depth of each treatment plot. The soil core data included L_v and W_v . The MR and soil core data were collected, based on plant phenological development (double ridge, early boot, milk, and soft dough; Zadoks et al., 1974), on four sampling dates. Double ridge, early boot, milk, and soft dough stages were days of the year 32, 91, 116, and 138 for Hunter, respectively; 60, 96, 125, and 141 for Stacy, respectively; and 53, 96, 116, and 144, respectively for Florida 302. An analysis of variance (ANOVA) (SAS Institute 1985, pp. 113–137) was performed to evaluate the effects of cultivar, sampling date, and soil depth on the dependent variables L_v , W_v , C_a , and L_a . A general linear models procedure was used to perform multiple regression and Pearson correlation coefficients calculation (SAS Institute 1985, pp. 433–506).

Peanut study

Using the procedure described by Box et al. (1989), minirhizotron tubes were installed in a Tifton fine loamy sand (fine loamy, siliceous, thermic Plinthic Paleudult) at the Coastal Plains Experiment Station, Tifton, Georgia (Meisner, 1990). Each of the plots were 12.2 m $\times$ 12.2 m. On 8 May 1989 Florunner peanut seed was planted in two rows on 1.83 m wide beds at 1 seed per 25 mm of row space. By 9 days after planting (DAP) the tubes were placed in the interior rows of each plot in two sets of four, with each tube spaced 2 m apart within the row. The angle of each tube was 1.05 rad from the soil surface and 0.228 rad and 0.13 m from the horizontal row. Plots were fertilized and maintained using recommended cultural practices (Johnson et al., 1987). In addition to a well watered control treatments were 30 day periods of water stress beginning 20 DAP, 50 DAP, and 80 DAP. Soil water in the well watered treatment was maintained throughout the study in the 0.20 to 0.60 m soil depths at 0.03 MPa or less. The other soil water treatments were kept at the same soil water status as the well watered control, except during the 30 day drought period when rainfall was excluded with a portable rainout shelter. Immediately following the drought period, soil water was restored by irrigation to equal the well watered conditions. Automatic portable rainout

Plot:____. Minirhizotron number:____. Depth:____.

			TIME PERIOD										
			i	j	1	2	3	,	,	j	,	,	n
T I M E P E R I O D	1			G_{11}	d_{12}	d_{13}	,	,	d_{1j}	,	,		n_{1n}
	2				G_{22}	d_{23}			d_{2j}				d_{2n}
	3					G_{33}			d_{3j}				d_{3n}
	,						$G_{,,}$		$d_{,,}$				$d_{,n}$
	i							G_{ij}	d_{ij}				d_{in}
	,								$G_{,,}$	$d_{,,}$			$d_{,,}$
	m										G_{mm}		d_{mn}

Fig. 1. Sample data sheet for analysis of recorded video tapes of root images from minirhizotrons. Note: i and j are both designated for time period between two recordings; G_{ij} =newly grown roots during time period i ; d_{ij} =roots which appeared during time period i and died during time period j . (Fig. 1 and caption from Cheng et al., 1991).

shelters described by Kvien and Branch (1987) were positioned near the plots to travel onto the plots when rain was detected during each stress period. On seven day intervals, starting at 15 DAP and continuing until 125 DAP, video recordings were made. Video recordings were analyzed by tracing root length images from a video display tube onto a sheet of clear plastic. Roots previously present, new roots, and old roots present at the last recording but not present at the subsequent recording were traced.

Data were analyzed as described by Cheng et al., 1991:

$$D_j = \sum_{i=1}^{i=j} D_{ij} \quad i=1,2,3,\dots; j=1,2,3,\dots \quad (1)$$

$$LR_j = \sum_j^{j=1} (G_{jj} - D_j) \quad j=1,2,3,\dots \quad (2)$$

$$DR_j = \sum_j^{j=1} D_j \quad j=1,2,3,\dots \quad (3)$$

$$TP_j = \sum_j^{j=1} G_{jj} \quad j=1,2,3,\dots \quad (4)$$

$$SGR_j = (G_j/t_j) / [(LR_{j-1} + LR_j)/2] \quad j=1,2,3,\dots \quad (5)$$

$$SDR_j = (D_j/t_j) / [(LR_{j-1} + LR_j)/2] \quad j=1,2,3,\dots \quad (6)$$

$$TI_j = (SGR_j + SDR_j) / 2 \quad j=1,2,3,\dots \quad (7)$$

where D_{ij} designates the roots present during the time i and died during time period j ; LR_j is the standing live root on the surface of the minirhizotron during time j ; G_{jj} is the root grown during the time period j ; DR_j is the total dead roots produced by time period j ; TP_j is the total root production until time j from the beginning; t_j is the time length during period j ; SGR_j is the specific root growth rate during time period j ; SDR_j is the specific root death rate during time period j ; and TI_j is the root turnover index during time period j .

A practical demonstration of MR root image data reduction is demonstrated in Fig. 1.

RESULTS AND DISCUSSION

These data indicate that wheat root data from MR have a good statistical relationship with wheat root data obtained by washing roots from soil cores. And that these data can be used to predict root weight, or root length density over the growing season. Similar conclusions can be drawn for the peanut plant and use of the minirhizotron data for determining root turnover index.

Wheat study

C_a and L_a of roots present per unit area from MR were evaluated in relation to L_v and W_v per unit volume of roots washed from soil cores. Analysis of variance (Table 1) shows that L_v , W_v , C_a , and L_a were each significantly influenced by sampling date and soil depth; however, only C_a was significantly influenced by cultivar. Multiple regression for L_v , W_v , C_a , and L_a with parameters of intercept, cultivar, sampling date, and soil depth were calculated. C_a is the only regression equation that has all parameters statistically significant. The multiple regression model correlation coefficients range from 0.88 for C_a to 0.69 for W_v . Thus, the C_a multiple regression equation is the best and can be used to make predictions of C_a as influenced by soil depths, climate, and wheat cultivars (Table 2).

Linear regression of L_v , W_v , or L_a on C_a provides intercepts that are highly significant except for W_v , and slope values that are all highly significant. The linear regression correlation coefficients for L_v , W_v , C_a , and L_a range from 0.69 to 0.94. Root count has correlation coefficients of 0.83, 0.69, and 0.72 for L_a , W_v , and L_v , respectively.

Thus, wheat root count data from MR can be reliably used to predict L_v , W_v , and L_a . Fortunately, measuring C_a is the least time-consuming measurement. Unfortunately, the prediction must be calibrated for each soil, climate and cultivar.

TABLE 1

Analysis of variance for root length density (L_v), root dry weight (W_v), root count (C_a), and root length (L_a)

Source	d.f.	Mean square	PR > F	r^2	C.V. (%)	Mean standard error
L_v (cm cm ⁻³) ^a						
Model	9	23.63	0.0001	0.81	41.99	0.54
Error	170	0.29				
Cultivar	2	0.57	0.1449			
Sampling date	3	5.20	0.0001			
Soil depth	4	49.00	0.0001			
W_v (mg cm ⁻³) ^a						
Model	9	0.99	0.0001	0.79	57.82	0.12
Error	170	0.01				
Cultivar	2	0.01	0.4786			
Sampling date	3	0.06	0.0094			
Soil depth	4	2.18	0.0001			
C_a (counts cm ⁻²) ^b						
Model	9	1.93	0.0001	0.84	40.36	0.14
Error	170	0.19				
Cultivar	2	0.14	0.0008			
Sampling date	3	0.24	0.0001			
Soil depth	4	4.09	0.0001			
L_a (cm cm ⁻²) ^b						
Model	9	2.14	0.0001	0.76	47.69	0.19
Error	170	0.04				
Cultivar	2	0.02	0.6263			
Sampling date	3	0.27	0.0001			
Soil depth	4	4.61	0.0001			

^aDetermined by washing roots from soil cores.

^bDetermined from root measurements on the surface of minirhizotrons.

Peanut study

The results presented in this section demonstrate the utility of the miniature video camera and minirhizotron for studying peanut root dynamics. TI index (eq. 7) is highest during the 15 to 60 DAP period and does not appear to be affected by drought stress treatment (Fig. 2). The interesting point in these data is that root TI was not appreciably influenced by imposing a thirty day drought stress at various time periods during the growth of otherwise well watered peanut plants.

TABLE 2

Multiple regression of dependent variable root count¹ (C_a) on the independent variables cultivar (X_1), sampling date (X_2), and soil depth (X_3) for the expression $C_a = a + bX_1 + cX_2 + dX_3$ ²

Parameter ³	Estimate	PR > T	Standard error of estimate	PR > F	r ²	C.V. (%)	Mean standard error
Model				0.0001	0.77	47.68	0.1638
Intercept (a)	0.9894	0.0001	0.04948				
Cultivar (b)	-0.0005	0.0023	0.00015				
Sampling date (c)	0.0272	0.0137	0.01092				
Soil depth (d)	-0.2073	0.0001	0.00863				

¹Viewed growing adjacent to a minirhizotron surface.
²Multivariate analysis of variance: regression procedure. SAS/STAT Users Guide 6.03, pp. 555, 850. SAS Institute. Cary, NC.
³Cultivar (X_1) = 100, Stacy; 200, Hunter; or 302, Florida 302. Sampling date (X_2) = 1, double ridge; 2, early boot; 3, milk; or 4, soft dough stage. Soil depth (X_3) = 1, 2, 3, 4, or 5, which are 0-15, 15-30, 30-45, 45-60, and 60-75 cm, respectively.

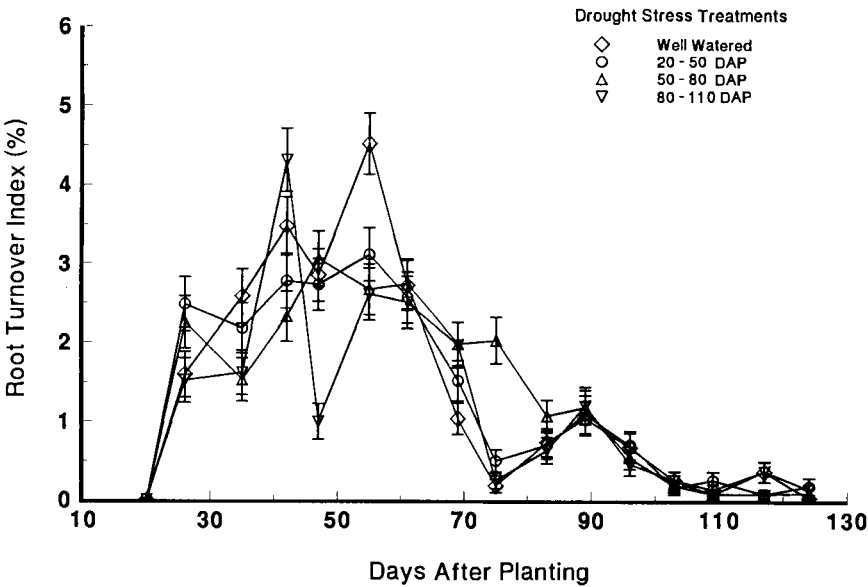


Fig. 2. Drought stress effects on peanut root turnover index.

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Clay– or sand–polysaccharide associations as models for the interface between micro-organisms and soil: water related properties and microstructure

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ABSTRACT

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Many soil micromicroorganisms are able to produce extracellular polysaccharides (EPS). Electron microscopic observations of soils demonstrated that EPS are produced in soils and are closely associated with the surrounding clay particles. In the present study, experimental clay–polysaccharide associations were taken as models for the soil/biota interface, and their microstructure and physical properties were investigated.

In the methodology, special attention was given to control the water potential and to preserve, as far as possible, the organizations of the original hydrated conditions.

EPS increased the water retention of clay minerals or sands on desiccation and on rehydration, and reduced desiccation and rehydration rates. This was explained by the strong water-holding properties of EPS.

Cryo-scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used, and showed that the polysaccharides occurred as a network of strands in the interparticle porosity of clay minerals. Polysaccharides changed the clay microstructure into an organo-mineral network with extensive interparticle bridging.

The present results show that the clay–polysaccharide associations exhibit specific physical properties and microstructures that should affect biological functions and survival, for example through storage of water and buffering against water potential fluctuations. Clay–polysaccharide sheaths also take part in the binding of aggregates by soil biota.

INTRODUCTION

Many micro-organisms produce extracellular polysaccharides (EPS), at least in pure culture, which can be firmly localized around the cell wall in the

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case of capsules; or occur as loose slimes that diffuse into the surrounding medium. In soils, when observed by electron microscopy, most micro-organisms are found to be surrounded by a sheath of amorphous material, that can be specifically stained as polysaccharides (Kilbertus et al., 1979; Foster, 1981). This sheath is in turn coated with clay minerals that are impregnated by EPS.

Since EPS and clay minerals are found to encapsulate most soil micro-organisms, it is important to determine whether such a micro-environment may affect the functions of microbes.

Among the physiological functions that have been proposed for EPS, emphasis has been directed towards their possible role in protecting microbes against desiccation and water potential fluctuations (Bitton et al., 1976; Kilbertus et al., 1979, Hartel and Alexander, 1986; Roberson and Firestone, 1992). It was suggested that polysaccharides could act as reservoirs for water in drastic water potential conditions, or as buffers that would slow the rate of drying of soil microbes (Dudman, 1977), but there is little direct experimental evidence of it. Actually, the survival of soil bacterial strains subject to desiccation stresses has not been clearly related to their ability to produce EPS in pure culture (Bitton et al., 1976; Osa-Affiana and Alexander, 1982a; Schmit and Robert, 1984; Hartel and Alexander, 1986). However, desiccation was found to stimulate EPS production by a soil pseudomonad (Roberson and Firestone, 1992).

A similar protective role was proposed for clay minerals, their ability to enhance bacterial survival to drying is well documented (Peña-Cabriaes and Alexander, 1979; Hartel and Alexander, 1986). As for EPS, clay minerals were considered to hold water as a reserve at low water potential (Peña Cabriaes and Alexander, 1979), to slow the rate of drying (Osa Affiana and Alexander, 1982b) or to allow a more thorough desiccation of bacterial cells (Bushby and Marshall, 1977).

The actual EPS concentrations in the vicinity of soil bacteria or fungi are unknown, but are probably quite high. If one compares for example the abundance of EPS strands around microbes when observed in soils or clay materials by cryo-scanning electron microscopy (SEM) (Dorioz and Robert, 1987) with model clay-polysaccharide associations of known concentrations observed with the same method (Chenu, 1989), it appears that the EPS content in the vicinity of microbes may far exceed 1% w/w of the mineral. Since it is not possible to separate natural clay or mineral-EPS associations that occur in the vicinity of soil microbes, in the present work, experimentally prepared clay-polysaccharide associations or sand-polysaccharide associations were taken as models for the micro-environment of microbes. The microstructure of associations and water-related properties that may be significant to microbes were examined, e.g., water retention at different water potentials, and kinetics of dehydration and rehydration.

MATERIALS AND METHODS

Minerals

The clay minerals consisted of kaolinite (St Austell, used without purification), or montmorillonite (Wyoming, purified and prepared in Ca form as previously described, Chenu, 1989). Quartz sand from Fontainebleau (France) was washed with 0.1M HCl and sieved 100–200 μm .

Polysaccharides

Various extracellular polysaccharides were used: scleroglucan, from the fungi *Sclerotium glucanicum* (Actigum CS11, CECA, France); xanthan, from the bacteria *Xanthomonas campestris* (Rhodopol, Rhone Poulenc); dextran from the bacteria *Leuconostoc mesenteroides* (Dextran 2000, Sigma chemicals). A purified EPS from *Rhizobium japonicum* was a gift from N. Amarger (INRA, Dijon).

Clay- or sand-polysaccharide associations

Mineral-EPS associations were prepared by mixing 2 to 5 g/kg aqueous solutions of EPS with 25 to 100 g/kg clay suspensions or with dried sand. In some experiments simple associations, i.e. mixtures, were prepared, whereas in others the excess of non-adsorbed polysaccharide EPS was discarded by centrifugation, and the term complex was then used. Kinetics and adsorption isotherms had been previously determined (Chenu et al., 1987, and unpubl. results). The selected polysaccharide contents were 1% w/w mineral for sand, from 1 to 10% for kaolinite (maximum adsorption levels are of 0.2% w/w mineral with dextran, 2.5% with xanthan and 4% with scleroglucan), and from 1 to 14% for Ca-montmorillonite (maximum adsorption is 14% w/w mineral with scleroglucan). NaN_3 5 ppm was used to prevent microbial contamination.

Water potential control and water content measurements

Portions of the clay-EPS or sand-EPS slurries, corresponding to about 0.5 g of dried montmorillonite or 1 g dried kaolinite or sand, were concentrated to cores under 0.032 MPa air pressure in a filtration device (Tessier and Berrier, 1979). The samples were then equilibrated with different water potentials (Ψ) using the same filtration device for -0.001 MPa to -0.1 MPa, pressure plates for -0.1 MPa to -2 MPa, and desiccators with saturated saline solutions for (-25 MPa to -100 MPa). The time required for equilibrium with applied water potential was previously determined as being 5–7

days in the filtration apparatus and pressure plates, and 3 weeks in desiccators. Once equilibrium was obtained, water content and apparent volume of the samples were measured by immersion in kerosene (Monnier et al., 1973).

In order to determine the water retention curve of pure EPS, EPS solutions were first concentrated to -0.4 MPa against polyethylene glycol (PEG 20,000; MW $\approx 20,000$, 150 g/l) (Williams and Shaykewitch, 1969). Samples corresponding to 0.1–0.2 g dried polysaccharide were then equilibrated with different water potentials using the above methods or PEG 8000. With PEG 8000 water potentials of -0.1 MPa and -0.5 MPa were obtained with solutions at respectively 74.1 and 166.6% w/w solutions (Michel, 1983).

For desiccation kinetics, cores were previously equilibrated with -0.032 MPa then allowed to dry in a chamber at 20°C at approximately 70% RH. Rehydration kinetics were determined with cores equilibrated with 0.032 MPa, air dried (-100 MPa), and then allowed to rehydrate in filtration devices under 0.001 MPa gas pressure for various times.

Examination of microstructure

Clay–EPS associations were observed with SEM and transmission electron microscopy (TEM). In order to preserve the organization in wet state specific preparation procedures were used.

SEM was performed with low-temperature techniques: millimeter size samples were rapidly frozen by immersion in melting nitrogen (-200°C), then introduced in the cryochamber of the microscope (Hexland cryotransfer CT10000). There, it was fractured at -180°C , superficially sublimated at -80°C , coated with gold at -180°C , and then introduced in the refrigerated column of the microscope to be observed at -180°C with a Philips SEM.

For TEM the samples were included in Spurr resin after progressively replacing water by solvents (Chenu and Jaunet, 1990). Ultrathin sections (50–80 nm) were made with a Reichert Ultracut and were specifically stained for polysaccharides with periodate oxidation followed by reaction with silver proteinate (Thierry, 1967). Samples were observed with a Philips EM 420 at 120 keV.

RESULTS

Water retention as a function of water potential

The main feature that could be observed in the water release curves of montmorillonite (Fig. 1a), kaolinite (Fig. 1b) or sand (Fig. 1c) was an increase of water contents in EPS-amended samples, especially at high water potentials (> -0.1 MPa). Increases were different among the polysaccharides with the following trend: the largest water contents were recorded with

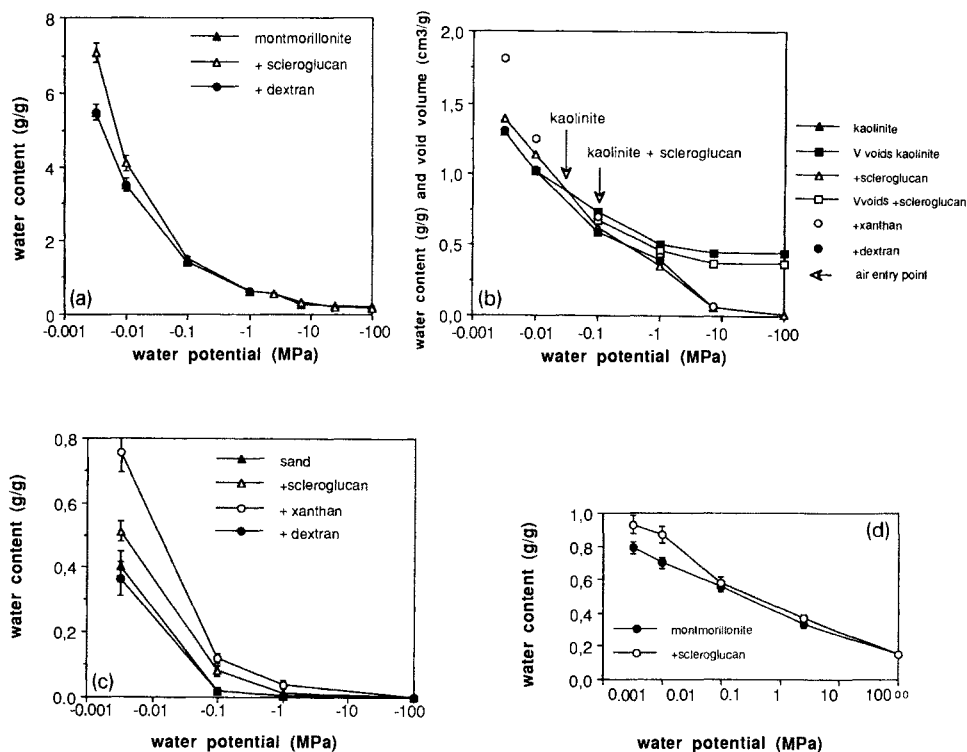


Fig. 1. Water retention curves of mineral-polysaccharide associations. (a) Ca-montmorillonite; EPS contents corresponds to maximum levels of adsorption: scleroglucan 14% w/w mineral, dextran 2%. (b) Kaolinite; EPS contents correspond to maximum levels of adsorption: scleroglucan 4% w/w mineral, xanthan 2.5%, dextran 1.5%. (c) Sand; EPS contents are 1% w/w sand. (d) Montmorillonite, rehydration from -100 MPa, scleroglucan content is 14% V voids is volume of voids. Results are the means of 5 replicates.

xanthan > EPS rhizobia > scleroglucan, whereas very little or no changes in water retention were obtained with dextran. The water retention increased with amount of added polysaccharide, as shown in Table 1 for scleroglucan at -0.0032 MPa. When water contents were compared at a given value of Ψ (-0.0032 MPa) and a given EPS content (1% w/w mineral), it was apparent that the lower the water holding properties of the mineral, the higher the increases in water retention due to EPS. Maximum changes in water contents were observed with sand; for example, its water content increased by 27% with scleroglucan (Table 1) and nearly doubled with 1% xanthan (Fig. 1c). Enhanced water uptakes were also recorded in rehydration after air drying of montmorillonite (Fig. 1d).

Measurement of the apparent volume of samples for kaolinite and montmorillonite with scleroglucan allowed the calculation of volume of voids per gram of solid by subtracting from it the volume of solid (Fig. 1b, kaolinite, data not shown for montmorillonite). The clay minerals have known densi-

TABLE 1

Water contents of mineral–scleroglucan associations at -0.0032 MPa

Polysaccharide content (% w/w mineral)	Water contents (g/g)		
	Sand	Kaolinite	Montmorillonite
0	0.4	1.3	4.47
1	0.51 (+ 26%)	1.31 (+ 1%)	5.7 (+ 4%)
4		1.46 (+ 12%)	
5			5.8 (+ 5%)
10		1.79 (+ 38%)	
14			7.23 (+ 31%)

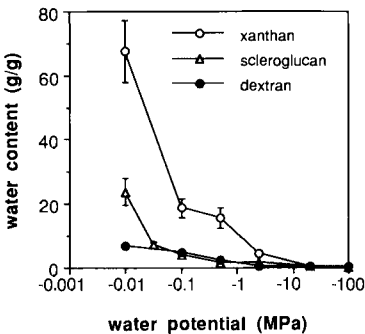


Fig. 2. Water retention curves of pure extracellular polysaccharides. Results are the means of 3 replicates.

ties of solid (kaolinite: 2.63, montmorillonite: 2.65), and for scleroglucan a solid density of 1.42 was taken (Bluhm et al., 1982). It appears that the air entry point with montmorillonite was not significantly changed (Chenu, 1989), while in kaolinite it was displaced from -0.01 MPa to -1 MPa at least (the volume of scleroglucan was certainly underestimated as the solid density of 1.42 given by Bluhm et al. (1982) only refers to the more organized and densely arranged portions of the polymer).

Higher water retentions in EPS-amended clays or sands seemed to be explained by the water held by the EPS themselves. The water retention curves of pure polysaccharides showed that they can hold very high amounts of water, more than 50 g water/g of polysaccharide (Fig. 2). The previously observed order in water retention, e.g., xanthan > scleroglucan > dextran persisted but only for high Ψ ; amounts of water held at low Ψ were more similar. Data were not obtained for EPS of *Rhizobium* since this polysaccharide was only available in very small quantities.

Kinetics of desiccation and rehydration

Desiccation kinetics were measured on kaolinite amended with a fairly high amount of polysaccharide, 10% w/w clay, and on sands with 1% EPS; all samples were initially equilibrated to -0.0032 MPa. For kaolinite, the water contents remained higher with EPS than in the pure clay during the whole desiccation process (Fig. 3a), although, the rates of loss of water (slope of the curve) were not very different with EPS. The water contents of these associations in equilibrium with -0.1 or -1 MPa were determined and are located, approximately, on the desiccation curves, as indicated by arrows (Fig. 3a). The rates of Ψ decreases were lowered with EPS present. In kaolinite the water potential decreased to below -0.1 MPa in 8 h, whereas it took about 15 and 25 h respectively with scleroglucan and xanthan to reach this water potential. Similarly, a value of Ψ of -1 MPa was attained within 15 h in the pure clay and only after 20 and 40 h with scleroglucan and xanthan. With sand the same trends were observed, although the rate of water loss also noticeably decreased in the presence of polysaccharides (Fig. 3b). Low water potentials were reached later in sand-EPS samples, e.g., it took 8 h to decrease the water potential to -0.1 MPa in the sand, between 10 and 15 h with dextran and 15–20 h with scleroglucan and xanthan.

The sudden rewetting of kaolinite from -100 MPa to -0.001 MPa was characterized by a very rapid water uptake and resulted in much higher water contents than in a desiccation process (Fig. 1b). With xanthan or scleroglucan at high contents, rates of water-uptake were lower and the final water content was also reduced (Fig. 4). With dextran or scleroglucan at 1% w/w, rates of water uptake were unaffected but the final water contents were low.

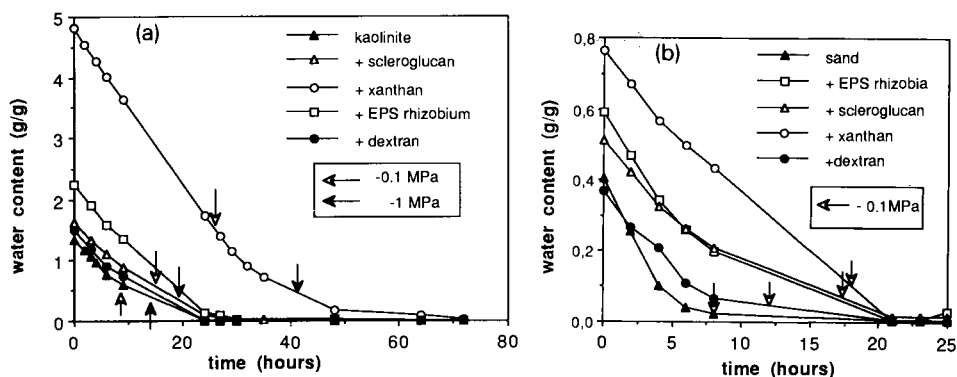


Fig. 3. Desiccation kinetics of mineral-polysaccharide associations. (a) Kaolinite; EPS content is 10% w/w clay, results are the means of 5 replicates. Water contents that correspond to water potentials of -0.1 MPa and -1 MPa are indicated with arrows on the desiccation curves. (b) Sand; EPS content is 1% w/w sand, results are the means of 5 replicates. Water contents that correspond to water potentials of -0.1 MPa are indicated with arrows on the desiccation curves.

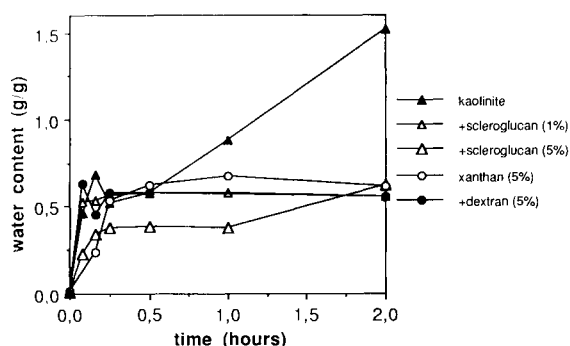


Fig. 4. Rehydration kinetics of kaolinite and kaolinite polysaccharide associations. Results are the means of 2 replicates.

Microstructure

The fabric of clay minerals consists of an arrangement of μm sized particles, rigid cristallites in kaolinite and thin and wavy quasicrystals in montmorillonite (Tessier, 1990). At high water potentials the interparticle pores are 0.5 to 4 μm in diameter and most of the water held is accommodated in such pores. When EPS were adsorbed, or added in excess of adsorption, to clays rather similar arrangements of clay particles were observed (Fig. 5), as previously reported for scleroglucan (Chenu, 1989). Strands of scleroglucan (Fig. 5b,c, e) or xanthan (Fig. 5d), of 0.1 to 1 μm length, were observed attached to the clay surfaces. With increasing EPS contents the pores were filled with a complex network of interconnected strands (Fig. 5c). Similar features were observed by TEM for both scleroglucan (Fig. 5f) and xanthan (data not shown). Pore diameters of the network were on average 0.2 μm when observed by SEM and 0.1 μm by TEM. With dextran strands were never observed in SEM.

DISCUSSION

Water holding properties of EPS

The polysaccharides used in this study held very high amounts of water, much more than clay minerals. Polysaccharides are known for their very high affinity for water. Generally, water retention by macromolecules at low water potentials are ascribed to adsorption of water (by hydrogen bonding, electrostatic interactions, etc.), whereas water held at high Ψ also involve capillary forces in large three-dimensional structures. The latter probably explains the high water retention at high Ψ by xanthan and scleroglucan, which have network structures (Morris and Norton, 1983; Stipanovic and Giammateo,

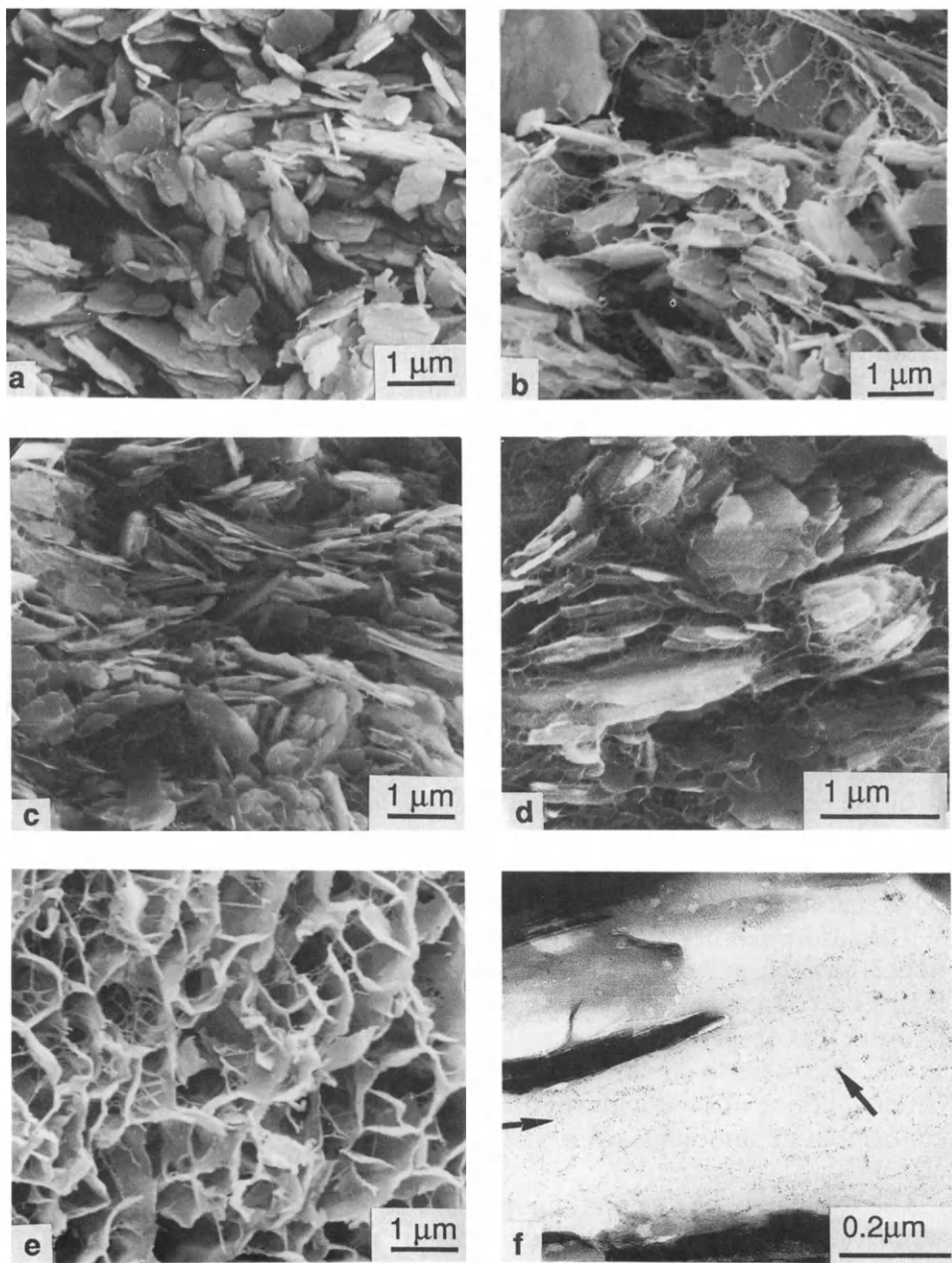


Fig. 5. Microstructure of clay-polysaccharide associations. (a) Cryo-SEM kaolinite, -0.0032 MPa. (b) Cryo-SEM kaolinite-xanthan; EPS content 2.5% w/w clay, -0.0032 MPa. (c) Cryo-SEM kaolinite-scleroglucan; EPS content 4% w/w clay, -0.0032 MPa. (d) Cryo-SEM kaolinite-scleroglucan; EPS content 10% w/w clay, -0.0032 MPa. (e) Cryo-SEM Ca-montmorillonite-scleroglucan; EPS content 14% w/w clay, -0.0032 MPa. (f) TEM kaolinite-scleroglucan; EPS content 4% w/w clay, -0.0032 MPa. Staining of EPS by silver grains is indicated by arrows.

1989). On the contrary, less water retention by dextran in this range of potentials could be in relation with the known coiled conformation and absence of networks (Morris and Norton, 1983).

When observed by EM techniques, xanthan and scleroglucan appeared as strands or, at high EPS contents, as networks of fibres. EM preparation techniques are liable to introduce artefacts such as an aggregation of polymeric chains due to the ice exclusion process in cryo-techniques (Kellenberger, 1987). It may be noted that (i) similar strand dimensions were obtained in the present study with two different techniques: freezing following by partial sublimation and water replacement by solvents. Strands were somehow thicker in SEM. (ii) Strands dimensions are compatible with the molecular dimensions. For example the elementary structure of scleroglucan is a triple helix of 35 Å diameter, not including the side chains (Bluhm et al., 1982). If a molecular weight of 1.5×10^6 is assumed, rods of 0.1–0.35 µm length can be built. In addition, aggregation of rods occurs in aqueous solutions (Stipanovic and Giammateo, 1989). (iii) Freezing artefacts are limited in concentrated polymeric systems (Kellenberger, 1987).

Modifications of mineral properties by EPS

The polysaccharides studied here were all adsorbed to clay minerals and sand (Chenu et al., 1987, and unpubl. data). Their adsorption to, or incorporation into minerals modified the microstructure and physical properties of the latter.

In the presence of EPS a general increase in water retention was found in the present study. This can be explained by both the addition of the water holding properties of EPS to those of the minerals, and the creation of a more open spaced arrangement of clay particles with EPS.

The adsorption of polysaccharides to clay minerals and the self-association of polymer chains led to the bridging of clay particles by polymer strands. At high EPS contents a real enmeshment of the clay particles into an organic network was observed. Such a bridging, visualized in the present study, was shown elsewhere to confer strong water stability to clays (Chenu, 1992). A better cohesion of sand with xanthan and scleroglucan was also noticed, and confirmed by modulus of rupture measurements (Chenu and Guérif, unpubl.).

The reduced rates of rewetting in kaolinite that were observed with polysaccharides in the present study are probably due to increased stability of the clay. High uptakes of water in kaolinite or illite upon sudden wetting have been reported (Tessier et al., 1992) and explained by slaking and extensive fissuration of the material (Tessier et al., 1990). With polysaccharides, particularly with scleroglucan and xanthan, slaking does not occur. Changes in wettability of the clay could also be involved.

EPS were found to have some similarities in microstructure and swelling

properties with smectites. Both can have three-dimensional networks and high water retentions. Changes in the physical properties of clays with polysaccharides were more drastic with kaolinite than with smectites (Chenu, 1992). Hence, the incorporation of EPS to soil minerals, as it occurs in the vicinity of microbes, can be expected to modify much more the properties of sands, silts, or clays with rigid particles, such as kaolinites, illites, vermiculites, than those of smectites.

Possible consequences for soil microbes

It has been proposed that the production of extracellular polysaccharides by soil micro-organisms may change the physical properties of the immediate environment of the microbe sufficiently to influence the functions and survival of microbes. This study was undertaken to assess to what extent the physical properties of minerals could be modified by EPS.

First, a stronger water retention was found with the addition of several EPS. This extra water may constitute a storage of water for microbes, as proposed by Dudman (1977), but mostly in a high water potential range (> -1 MPa) and only if microbes can effectively compete to extract this water. Higher water contents at a given Ψ would however facilitate the activity of extracellular enzymes and the diffusion of nutrients, as suggested by Roberson and Firestone (1992). Kaolinite samples desaturated at lower Ψ with polysaccharides, a phenomenon that is likely to be more pronounced for minerals with larger particle sizes. For microbes, it could mean that with EPS continuous saturated pathways for the diffusion of nutrients will exist at lower water potentials.

Desiccation rates of minerals were affected by EPS. Similar results have been reported by Roberson and Firestone (1992) for the desiccation of sands amended with the EPS of a soil *Pseudomonas*. In their study, as in the present one, the rates of water loss were less affected than the rates of water potential decrease. This means that extracellular polysaccharides can act as buffers during water potential fluctuations, and thus may give time to the micro-organisms for the production of osmoprotectant solutes and reduce the amplitude of water potential fluctuations experienced by the micro-organisms. During rewetting of soils, fluctuations in Ψ are usually more rapid than during desiccation, and rewetting has been found to be a very drastic event for soil microbes (Kieft et al., 1987). Rates and amount of water uptake in kaolinite during rehydration were also reduced by EPS, though it remained a very rapid process. Rate of Ψ increase could not be determined, though the slaking of pure kaolinite is probably associated with the presence of free water in the fissures, on the contrary to EPS amended samples.

CONCLUSION

Soil micro-organisms often produce extracellular polysaccharides that tend to form with the surrounding mineral particles an organo-mineral sheath around the cells. Model clay- or sand-EPS associations were found to have original physical properties, different from that of pure minerals, that may affect the functions of microbes in several ways. The present study confirms the assumption that EPS-mineral coatings of soil micro-organisms constitute a micro-environment of high ecological significance. This concept found its application with the use of polymers and organo-mineral associations as inoculant carriers for microbes (Jung et al., 1982; Fravel et al., 1985). Clay-polysaccharide sheaths of microbes also constitute an important contribution to the binding of aggregates by soil biota.

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Quantification of fungal morphology, gaseous transport and microbial dynamics in soil: an integrated framework utilising fractal geometry

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ABSTRACT

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The consequences of heterogeneous structure for nutrient acquisition by soil fungi, microbial dynamics and transport in soil are studied. Fractal geometry provides the unifying theme and forms the basis of a theoretical framework for studying dynamics in heterogeneous media. The interpretation of foraging strategies of soil fungi are presented which suggest that the processes governing branching and hyphal mass distribution are independent. Classical diffusion is shown to be inappropriate for the study of diffusion in heterogeneous soil and a new theory is proposed which incorporates heterogeneity and pore tortuosity. The consequences of structure for microbial spatial and temporal dynamics are examined and it is found that an understanding of these and related processes such as nutrient cycling must include the role of soil structure. While stressing the need to appreciate the relevance of the theory to any particular application, it is shown that quantitative fractal geometry can yield insights into the mechanism whereby spatial organisation influences the interaction between structure and biotic processes in the soil.

INTRODUCTION

A great many natural phenomena possess non-homogeneous structure on spatial scales which are relevant to the processes which concern them. This leads to difficulties in gaining a quantitative understanding of these phenomena since it is not possible to express meaningful and unique average measures regarding the processes. Since the magnitude of the measures depends on the scales at which they are measured, there exists no scale of interest at

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which the measures cease to be dependent on change and an average value can be defined. Fortunately, many such irregular structures occurring in Nature obey well-defined scaling laws. These laws relate to the rate of change of the measure with resolution of the measurement, and are a generalization of classical Euclidean geometry. The length of a line is defined as the length of the unit used to measure it, multiplied by the number of times the unit can be placed along its length. In Euclidean geometry, if the unit size is halved, twice as many are required to span a length and in general, the number of units $n(l)$ is related to size of the unit, l , by the relation:

$$n(l) \approx \frac{1}{(l/l_{\max})^D} \quad (1)$$

where D is the Euclidean dimension equal to one in this instance, and $l_{\max}$ is the largest length which spans the line. This relation extends to two and three dimensions when D is equal to two and three, respectively, and where l is the length of the side of tiles which tessellate a plane, or the edge of boxes which fill a volume. However, where we are concerned with a very large class of shapes which fill space with a smoothness intermediary to the Euclidean forms, we find we can generalise eq. (1) to hold for fractional values of D . Such shapes, which include clouds (Mandelbrot, 1983), plants (Peitgen and Saupe, 1988) and soil (Young and Crawford, 1991a; Bartoli et al., 1991), are known as *fractals* and D is the *fractal dimension*.

It is crucial to understand the relevance of the application of this geometry carefully before interpreting results. While the dimension as defined in eq. (1) is a useful characterization of certain measures on structures of different form, it is not a complete description and at best its interpretation in relation to function can be subtle. For example both a teacup and a saucer have dimension two (ignoring the thickness of the china) but clearly have quite different functional forms. Indeed for most fractals it is possible to make alternative, equally valid measures of dimension, each relevant to a slightly different property of the structure (Halsey et al., 1986). We will meet an example in the treatment of soil below. Such fractal shapes require an infinite number of these associated dimensions for a complete physical characterisation (Hentschel and Procaccia, 1983), although for most processes only a few measures are relevant to the investigation. In the example above, the dimension says nothing of the relative water-holding capacities of cup and saucer, but it does tell one about the amount of extra glaze required for a cup and saucer of twice the size.

Within a carefully defined quantitative framework, fractal analysis of complex structures can yield insight into processes which have hitherto proved inaccessible to experimental or theoretical insight. Used in this way, it is more than just a diagnostic tool. This paper is concerned with its use in understand-

ing the complex interrelations between soil structure, microbial dynamics and transport processes in soil.

THE GEOMETRY OF FUNGAL FORAGING

Fungi are an important component of the soil biomass involved in many nutrient cycling reactions. Many fungi also possess the ability to translocate substances within their colonies (Wilcoxson and Sudia, 1986; Jennings, 1987). This potential ability of fungi to short-circuit the physico-chemical constraints imposed on nutrient mobility makes it important to understand the mechanisms of nutrient acquisition and distribution within the colony. In turn this requires a functional understanding of the colony structure including the influence of resource distribution on morphology. Fractals have been used to describe the morphology of microbial colonies (Fujikawa and Matsushita, 1989; Matsuyama et al., 1989; Obert et al., 1990a, b) and have been interpreted in terms of a foraging strategy in a species of soil fungus (Ritz and Crawford, 1991). In the latter case, colonies of *Trichoderma viride* were grown on cellophane over a *homogenous* substratum. The fractal dimension was hypothesized to reflect the compromise between exploitative and explorative growth forms. It was suggested that when the nutrient status of the substratum was poor, the colonies formed a low-dimensional morphology, thus distributing as little hyphal mass as possible across a maximal area. Upon encountering a nutrient concentration, the dimension increases towards 2, filling space as effectively as possible in order to fully exploit the new source of substrate. The mechanism by which the dimension is raised is through increased levels of branching (Ritz and Crawford, 1991). In a colony of low dimension, there is more hyphal mass in large- rather than small-scale structure, compared with a colony of higher dimension where hyphal mass is more evenly distributed across scale. Although these ideas address the question of the adaptation of morphology to the nutrient status of the substrate, they do not attempt to study the influence of resource distribution on morphology. This is important in the context of the ability of fungi to redistribute a patchily distributed nutrient resource in the soil. We present here results of a study of the morphological response of *Trichoderma viride* to a heterogeneous nutrient distribution.

Sheets of cellophane (6×6 cm) were placed in sterile petri dishes and inoculated with single germinated spores of *Trichoderma viride*. The atmosphere in the dishes was kept humid by including a saturated paper pad in the dish. A discrete nutrient resource was placed in some dishes 2 cm away from the germling at the same time as inoculation. The resource comprised a cylinder of agar 1.8 mm diameter×2 mm high, containing 2% glucose, 0.2% NaNO₃ and 0.2% KH₂PO₄. Photographs of the colonies were taken at daily

intervals by direct projection of the colonies, using a photographic enlarger, onto lith film. Two examples are shown in Figs. 1 and 2.

In either colony there was very little growth in the direction opposite to that of the nutrient bait. In fact the growth in this direction was actually inhibited, being less than in the control plates where no nutrient bait was present. Secondly, in the half of the plate where the hyphae flourished, growth emanated from the point of inoculation to form a radially symmetric pattern. This is surprising if growth rate actually responds locally to the nutrient status of the substrate, since if nutrient is diffusing radially from the bait, the hyphal concentration profile should not be radially symmetrical about the point of inoculation. Hence if growth rate is a local response, the colonies should be elongated along the line joining bait to inoculum site. Finally, there was also

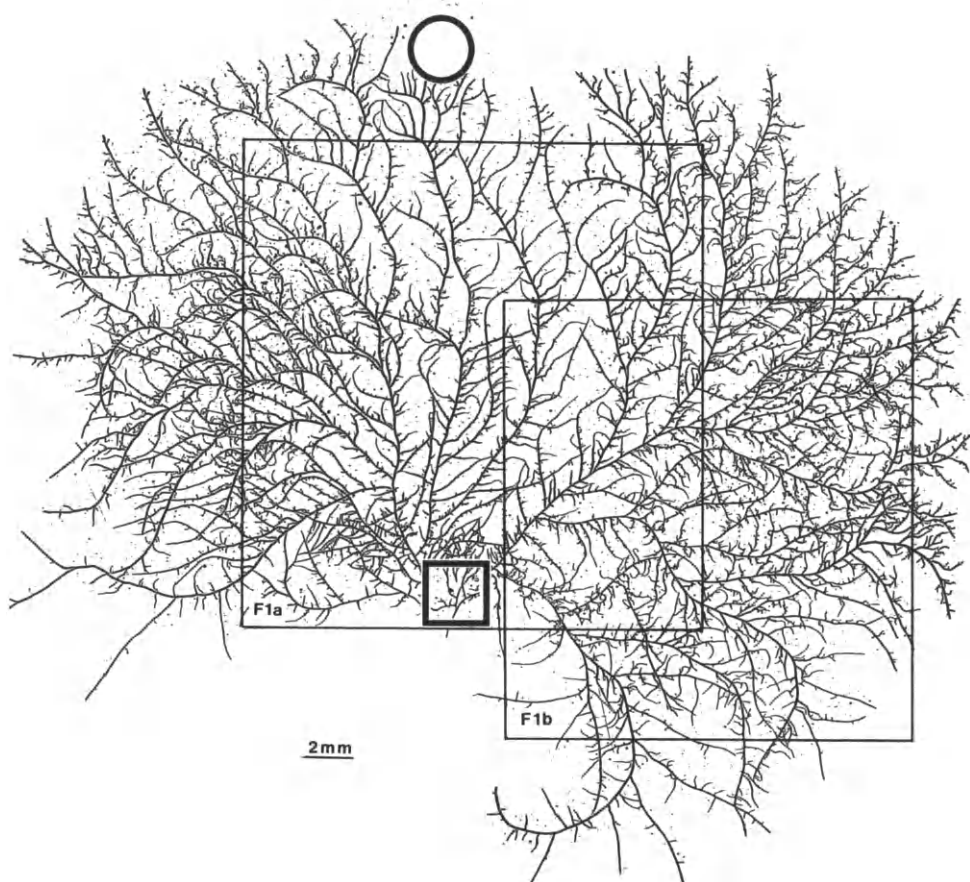


Fig. 1. Colony of *Trichoderma viride* grown on cellophane showing growth from inoculation site (small square) toward nutrient bait (small circle). The images are divided into the regions shown and the results of the analyses for each portion are shown in Table 1.

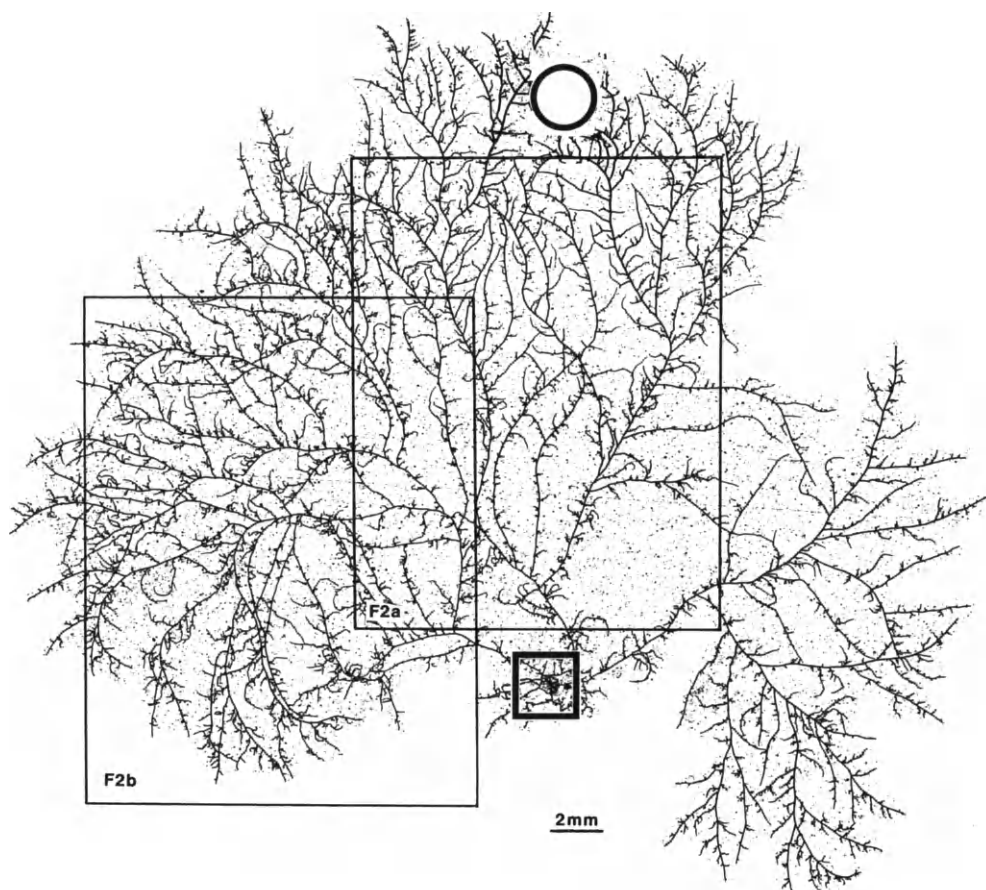


Fig. 2. See caption of Fig. 1.

an apparently greater concentration of hyphal mass in directions away from the nutrient bait in both colonies, an observation which apparently contradicts the hypothesis of Ritz and Crawford (1991). To quantify this assessment, we address the question of how the space-filling properties of the pattern adjust to the heterogeneous levels of substrate nutrition.

For the purpose of analysis, the images presented in Figs. 1 and 2 were each divided into two portions as indicated in the figures and each was digitized using a commercial scanner (Canon Microtech) to produce a 440×320 pixel grid. The portions were chosen to be representative of structure in the vicinity of the direction joining bait to site of fungal innoculation, and of the structure to one side of the colonies. The images were analysed by measuring the rate of increase of the number of pixels belonging to the structure with distance from a number of randomly chosen origins in the structure. If $N(r)$ is

the number of pixels belonging to the structure inside a region of size r , then the number inside a larger region of size R is simply $N(r)$ multiplied by the number of times the region of size r fits inside the region of size R . Therefore, using eq. (1), $N(R) = n(r)N(r) = (R/r)^D N(r)$, which has solution $N(r) \approx r^D$. The fractal dimension was therefore reduced from a plot of $\log N(r)$ vs. $\log r$. Such measurements were repeated in each portion for 600 randomly chosen origins and the average slope was calculated. The results for each portion are presented in Table 1. It is possible that the size and precise position of the portions will influence the quoted values of dimension and we are currently undertaking a detailed examination of this effect. However, the errors quoted in the table (reflecting the variation in the derived values of D for each origin position within a portion) are small, and so the effect on D of subtle changes in position of the portions are likely to be unimportant for our conclusions.

In both colonies the structure in the direction of the nutrient bait has a fractal dimension not significantly different from 2, while the structure away from this direction has a dimension significantly less than 2 ($P < 0.0001$). This observation is consistent with the hypothesis that the space-filling efficiency of each part of the colonies reflects the deduced local nutrient distribution. For both colonies, there is more hyphal mass (assuming constant mass/unit area) in directions away from the bait ($\sim 20\%$ more in F_{1a} compared with F_{1b} , and 5% more in F_{2a} compared with F_{2b}) despite the fact that the space-filling efficiencies are greater in directions towards the bait. This would seem to suggest that the processes controlling branching and the distribution of mass are independent. This conjecture is further supported by the observation that there is no growth in directions opposite to the bait. If the mass distribution was inversely related to nutrient status, then there should be more mass where nutrient concentrations are low, i.e., in the posterior zones of the membranes. Since a composite nutrient resource was used in this study, it is unclear which components elicited the responses. The experimental design would readily permit the effects of single component resources, such as energy rich compounds vs. inorganic nutrients on foraging strategies, to be studied. At present, we are conducting a range of experiments to test these

TABLE 1
Fractal dimensions of the hyphal patterns in regions defined in Figs. 1 and 2. Numbers quoted are the mean and the standard error

Region	D
F_{1a}	2.01 ± 0.0049
F_{1b}	1.97 ± 0.0034
F_{2ac}	2.00 ± 0.0079
F_{2b}	1.93 ± 0.0069

ideas. In the studies presented here, the colonies were constrained to grow in two dimensions, in order to facilitate the fractal analysis. We acknowledge that this is artificial, albeit in nature fungi do colonise surfaces as well as ramify in three dimensions through their substrata. However, for preliminary studies a three-dimensional approach would be prohibitively difficult and at present we can only conjecture that principles established by observing colony formation on a surface may be extrapolated to three dimensions.

By separating the phenomenon of mass distribution and space-filling efficiency objectively and quantitatively, fractal analysis offers a potentially powerful tool for understanding the morphological response of fungi to nutrient status. We shall see below that the approach will also lend itself to a treatment of transport in soil and to structural considerations in the spatio-temporal dynamics of soil microbes. This running theme holds promise of a unified treatment of the transport and cycling of nutrients in the soil.

TRANSPORT IN A FRACTAL SOIL STRUCTURE

In studying the biological, physical and chemical processes which take place in the soil environment, soil structure is at best treated qualitatively but more usually is ignored by assumption, or eliminated through artificial processing of aggregates. Yet structure is clearly important for governing the supply of oxygen, water and nutrient to plants and microbes, and the dynamics (spatial and temporal) of microbial populations. The slow rate of progress in the incorporation of structure into an understanding of the associated processes is due largely to the lack of an appropriate measure of heterogeneity. Recently, measurements have shown that the arrangement of soil particles (Bartoli et al., 1991; Young and Crawford, 1991a), pore space (Bartoli et al., 1991) and surface features (Young and Crawford, 1991b) can be adequately approximated by fractals over size scales from a few microns to several centimetres and above—scales which are clearly relevant to process concerning microbes, roots and chemistry. Despite early promise however, the difficulties in finding a functional interpretation of the measured values of dimension (Young and Crawford, 1991a) has limited progress. Part of the problem resides in the so-called “lacunarity” (Mandelbrot, 1983) of soil structure. While the fractal dimension may be related to how a measure on the structure scales with spatial resolution, the lacunarity is concerned with the variability of that measure at a given resolution. As an example, Fig. 3a, b shows the first two stages in the construction of a fractal known as a Sierpinski Carpet (Mandelbrot, 1983). The Carpets in both constructions have the same fractal dimension, but Fig. 3a has lower lacunarity than Fig. 3b. Clearly, the lacunarity, or the magnitude and arrangement of holes in a structure, may have a significant bearing on the processes which occur on that structure. Therefore the fractal dimension may not on its own be sufficient to uniquely define the heterogeneity of an object

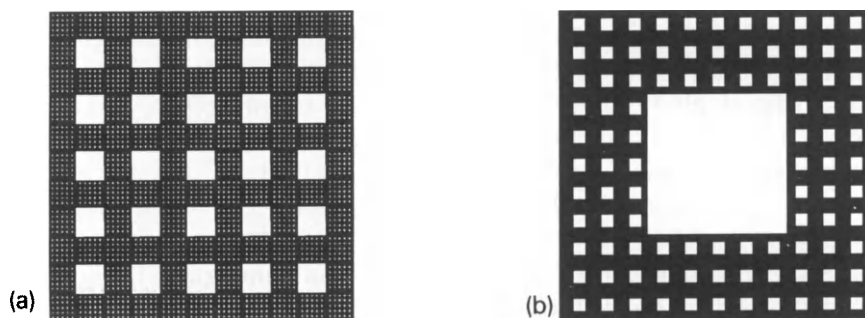


Fig. 3. The second stage in the construction of two Sierpinski Carpets (Mandelbrot, 1983) of identical fractal dimension: (a) has a lower "lacunarity" than (b) while both have a fractal dimension equal to 1.904.

for the purposes of understanding these processes. Depending on the nature of the problem, additional measures may be required and in relation to diffusion, one such measure is the fracton dimension (Orbach, 1986; Havlin and Ben-Avraham, 1987).

The mean squared displacement of a particle diffusing in free space is related to travel time by:

$$\langle r^2 \rangle \propto t^{2H} \quad (2)$$

where r is the distance of the particle from its starting point after time t and $H = 1/2$ in the familiar case of a Brownian random walk. However by altering the value of H , the character of the particle's trajectory can be changed. In particular, the tendency to turn back on itself is controlled by the parameter H . It can be shown (Mandelbrot, 1983; Peitgen and Saupe, 1988) that the parameter H is inversely related to the fractal dimension D_w , of the particle trajectory, i.e.:

$$H = 1/D_w \quad (3)$$

and therefore that the trajectory is more or less space filling depending on the value of H . One may anticipate that when the particle's motion is constrained to move in a fractal network, that there would be some connection between the space-filling properties of the trajectory, and the geometry of the space through which it is moving. In particular, if the network is particularly tortuous, then a similar tortuosity would be imposed on the trajectory of the particle mediated through a modification of the parameter H .

To derive the explicit connection, consider the instantaneous random scatterings of the particle from one point in space to the next in a tortuous fractal network. The number of distinct points visited is proportional to the volume of space explored, i.e.:

$$\text{No. distinct points visited} \sim R^{D_N} \quad (4)$$

where R is the straight-line distance of the particle from its starting point, and D_N is the fractal dimension of the network space. However, by definition of the fractal dimension of the trajectory:

$$R \approx N^{1/D_W} \quad (5)$$

and therefore if S_N is the number of distinct sites visited after N scatterings;

$$S_N \approx N^{D_N/D_W} \sim N^{\bar{d}/2} \quad (6)$$

where $\bar{d}$ is the fracton dimension (sometimes also referred to as the fraction dimension). Finally, since $H = 1/D_W$, eq. (2) can be rewritten:

$$\langle r^2 \rangle \approx t^{2H} \approx t^{\bar{d}/D_N} \quad (7)$$

Thus the constraints imposed by the tortuosity and heterogeneity of the network impress themselves on the rate of diffusion via a combination of both the fracton and fractal dimensions associated with the network. In free space and in three dimensions $D_N = \bar{d} = 3$ and we have classical Brownian motion. The main implication of these results is that the classical diffusion equation cannot be correctly applied to diffusion in soil and should be replaced by the form (Orbach, 1986):

$$\frac{\partial c}{\partial t} = \nabla \cdot (D(r) \nabla c) \quad (8)$$

where c is the concentration, and $D(r)$ replaces the constant diffusion coefficient where:

$$D(r) \sim r^{-\theta}; \theta = 2(D_N - \bar{d})/\bar{d} \quad (9)$$

If qualitative terms are to be attached to the interpretations of D_N and $\bar{d}$, then the heterogeneity or “clumpiness” of the pore network should be associated with the value of D_N , while the tortuosity or the degree to which the network impedes the forwards progression of a diffusing particle, should be associated with $\bar{d}$.

Following the method for the calculation of $\bar{d}$ suggested by eq. (6), measurements were made on thin sections of two soils with contrasting pore structures shown in Fig. 4a, b. The images were first digitised using a commercial scanner (Canon Microtech) and then reversed to produce a digital image of the pore space. A Monte-Carlo simulation was performed by assigning to each pixel a potential site of a randomly walking particle. At each iteration, the particle was assumed to move from one pixel to any one of up to eight pixels which surrounded it. If the particle reached the boundary of the image, the walk was terminated and a new one initiated at some random point in the image. Computations were based on the average of between 10^3 and 10^4 individual random walks each of up to 6×10^3 steps on a 440×320 grid. The

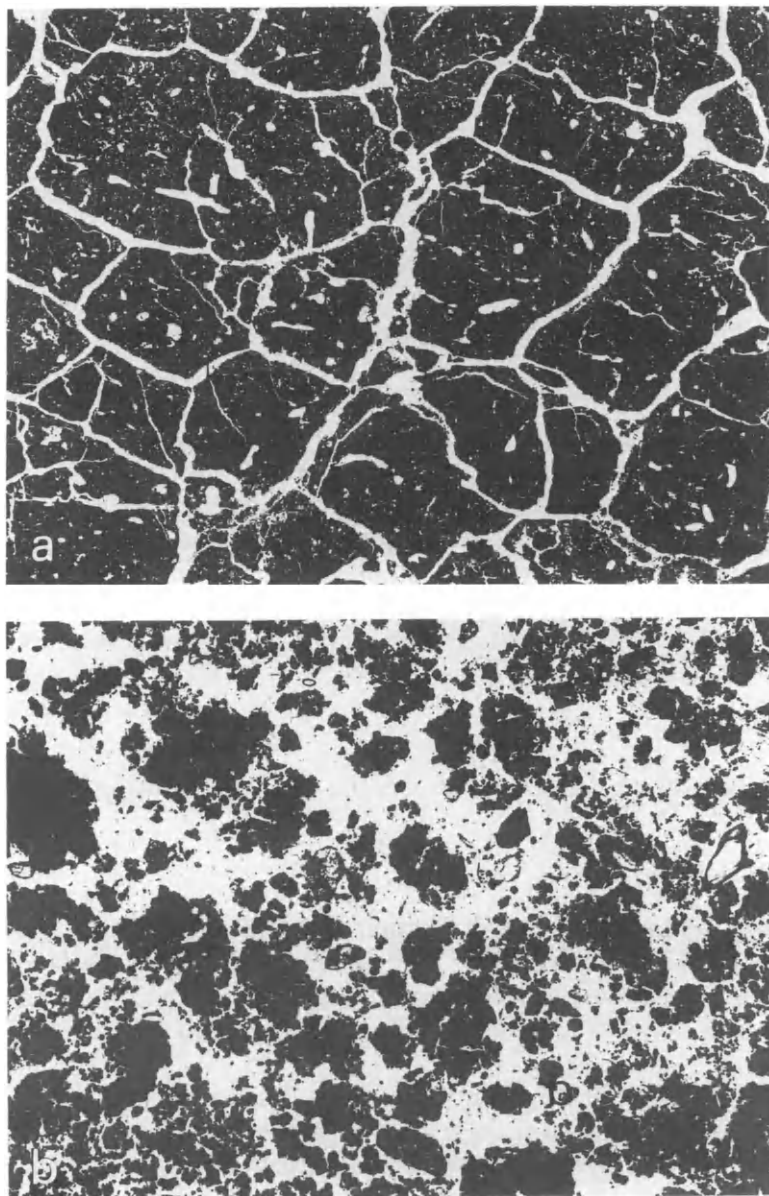


Fig. 4. Thin sections of soils of contrasting structure: (a) shows a soil of angular blocky structure while the soil in (b) has a crumb structure.

number of distinct sites visited as a function of the number of steps taken was recorded. A plot of $\log S_N$ vs. $\log N$ should yield a line of slope $\bar{d}/2$. As a test of the method, the simulation was performed on a regular fractal network known as the Sierpinski triangle (Mandelbrot, 1983) where, because of the

method of construction, an analytical derivation of the fracton dimension is possible (Haus and Kehr, 1987) and yields $\bar{d}/2 = \log_e 3 / \log_e 5 \simeq 0.683$. The value returned by the Monte-Carlo simulation was $\bar{d}/2 = 0.687 \pm 0.001$ in satisfactory agreement, given the caveat that the error is likely to be underestimated because S_N is a cumulative variable.

Since a full description of structure relevant to diffusion also requires the determination of the fractal dimension, D_N was calculated for the pore networks using the standard method (Peitgen and Saupe, 1988) of counting the number, $N(r)$, of pixels residing in pore-space within radius r of some origin in the network. Then, since:

$$N(r) \propto r^{D_N} \quad (10)$$

a linear fit through a plot of $\log N(r)$ vs. $\log r$ yields D_N . This method was applied to the digitized images, and averages for each soil were calculated from 200 randomly chosen origins. Table 2 shows calculated values of D_N and $\bar{d}$ for the two dimensional sections shown in Fig. 4a, b. Assuming that the soil structure is isotropic at the scales considered, the values of $\bar{d}$ and D_N can be extrapolated to three dimensions by adding 1. The corresponding values of θ and $2H$ are listed in Table 2.

Some interesting observations can be made regarding these results. Firstly, in both soils, the flow is sub-diffusive in the sense that $2H$ (eqs. 2 & 7) is less than 1 and therefore diffusing particles move out from their origin more slowly than under the assumption of classical (Brownian) flow. This will be true for all soils for which the fracton and fractal dimensions can be defined since it can be shown that $\bar{d} \leq D_N$ (Hentschel and Procaccia, 1983). Secondly, the heterogeneity of the pore network as defined by D_N , of soil A (Fig. 3) is greater than that of soil B (Fig. 3b). Finally, the tortuosity of the network in soil A is greater than that in soil B. However, despite the observation that both the tortuosity and heterogeneity of the pore-space of soil A are greater than in soil B, the rates of diffusive flow (as measured by H) are comparable. This apparent contradiction can be understood by careful consideration of the separate effects of heterogeneity and tortuosity on the diffusive flow. Heterogeneity, which increases as D_N falls below 3 (or 2 in two dimensions) has the effect of reducing the effect of dilution of the flow as it moves outwards from

TABLE 2

Values of D_N , $\bar{d}$, θ and $2H$ (see text) for the soil sections shown in Figs. 4a, b along with values of θ_{3D} and $2H_{3D}$ deduced for the soils in three dimensions

Soil	D_N	$\bar{d}$	θ	$2H$	θ_{3D}	$2H_{3D}$
Soil A	1.71	1.04	1.29	0.61	0.66	0.75
Soil B	1.94	1.33	0.92	0.69	0.5	0.79

its origin. This is because in a homogeneous network, the pore-space per unit volume is a constant, leading to a rapid drop off in concentration as particles diffuse from their origin. In contrast, when the network is fractally heterogeneous, the pore-space per unit volume available to the diffusing particles, decreases with distance from the particles' origin, thereby concentrating the flow by channeling the particles into a progressively rarefied network. On the other hand tortuosity, as measured by the deviation of $\bar{d}$ from 3 (or 2 in two dimensions) affects the spatial distribution of particles by retarding their forward movement. Therefore heterogeneity and tortuosity have competing effects on the rate of progression of diffusing particles, and in our two soils the differences between their respective heterogeneities and tortuosities cancel to impose more or less identical constraint on diffusive flow.

Whilst the diffusion of particles through an inert network is a relatively simple process to understand, the additional complications of chemical and biological interactions quickly make the problem less tractable. Even more so when the diffusion particles form a fluid and viscosity and surface tension effects are important. The question of relevant resolution is also an important one since clearly at higher resolution, pores which were previously unconnected may appear connected. To resolve this problem, it will be required to analyse the same section at a number of resolutions and deduce the asymptotic value of $\bar{d}$ as the resolution increases. At some resolution, the extra connectivity observed will be inconsequential from the point of view of gas transport, and the specification of this point will require experimental backup. The approach presented here is intended to form the foundation for a more realistic treatment, and as we shall see in the next section, it can provide insight into other processes such as movement of free-swimming microbes and the influence of habitat topology on microbial dynamics.

MICROBIAL DYNAMICS IN A FRACTAL HABITAT

One of the properties of a fractal terrain is that the measured value of total surface area depends on the scale of measurement. If the measurement is made at the resolution of a small microbe, then the measured area represents the habitable space available to species of that size. This concept has been used to relate body size to population density in fractal habitats (Morse et al., 1985; Marquet et al., 1990). In relation to the population dynamics of soil micro-organisms, the existence of refuge sites in soil, i.e., niches of small-scale structure accessible to bacterial species but which exclude predatory protozoa, has been invoked as an important factor in the persistence of the smallest prey species (Steinberg et al., 1987; Hattori, 1988; Postma and Van Veen, 1990; Heijnen and Van Veen, 1991). Furthermore, the mobility of these predators must be constrained by the sort of structural considerations outlined before. If the dynamics of cycling and transport of nutrients via the soil microbial

population is to be understood, considerations of these sorts must be taken into account.

Young and Crawford (1991b) present results indicating the fractal nature of fracture profiles in soil. Since bacteria tend to reside on the surface of pore walls where they may be predated by protozoa, these results enable the prediction of the magnitude of the area of refuge sites for small prey species of size r_1 from larger prey species of size r_2 . This in turn will give some insight into the fraction of the microbial population which is vulnerable to predation, and the influence of soil structure on that fraction. To calculate the fraction of the potential habitat of the prey species which is inaccessible to predators, we use the result (Mandelbrot, 1983; Peitgen and Saupe, 1988) that the resolved surface area $A(r)$ at resolution r is given by:

$$A(r) \approx 1/r^{D-2} \quad (11)$$

where D is the fractal dimension of the terrain. Identifying this with the habitat space available to a species of size r_1 , we find that the area of the refuge space of species 1 is given by:

$$A(r_1) - A(r_2) \approx \frac{1}{r_2^{D-2}} \left[\left(\frac{r_2}{r_1} \right)^{D-2} - 1 \right] \quad (12)$$

Hence the fraction $F_{1,2}$ of space available to species 1 which provides refuge from predation by species 2 is given by:

$$F_{1,2} = 1 - \left(\frac{r_1}{r_2} \right)^{D-2} \quad (13)$$

This implies that if species 1 is a bacterium ($r_1 \sim 5 \mu\text{m}$) and species 2 is a protozoan ($r_2 \sim 30 \mu\text{m}$), then for the Bindlach Meadow soil ($D=2.36$) in Young and Crawford (1991b) almost half of the potential habitable area of the bacteria is inaccessible to protozoan predation. This fraction increases when one considers the space interior to the surface of the soil. Clearly soil structure should have a very significant impact on microbial population dynamics.

This and the work of the previous section suggest that structure may also have a very significant influence on the spatial dynamics of microbes in soil. Indeed in a tortuous soil where a greater number of available diffusive pathways will exist to divert smaller microbes from an outward flow, structure will influence small microbes differently to large. To study the extent to which this is true, we consider the case of randomly moving microbes diffusing in a saturated soil. While mass flow and fluid films (e.g. Wallace, 1958) are likely to dominate in affecting microbial movement in soil, we attempt here to isolate the influence of structure. Measurements were carried out on the soil images in Fig. 4a, b to determine the sensitivity of diffusion rate to body size. Values of D_N and $\bar{d}$ were obtained by first assuming a minimal gap size of $100 \mu\text{m}$

through which a particular microbe ("species 1") can pass, and comparing these with results assuming a minimal gap size of $200\ \mu\text{m}$ ("species 2"). These measurements were made simply by reducing the resolution of the digitized images to 100 and $200\ \mu\text{m}$, respectively, and repeating the analyses of the previous section. Equation (7) is complete by inclusion of a multiplicative constant on the right-hand side which relates to the motility of a particular species. If we assume species 1 to initially move faster by virtue of a greater motility, but subsequently to diffuse more slowly because of the constraints of tortuosity, Then if both species begin at the same point in the soil there will be some time t_0 when species 2 overtakes species 1. This being the case we can derive a relation between the crossing times for each species to move a given distance in the soil. If t_1 is the time taken by species 1 to move a distance R from its starting point, then the time taken by species 2 to travel the same distance is given by:

$$\left(\frac{t_2}{t_0}\right) = \left(\frac{t_1}{t_0}\right)^\phi; \quad \phi = \left(\frac{D_2}{D_1}\right) \left(\frac{\bar{d}_1}{\bar{d}_2}\right) \quad (14)$$

For the soil A in Fig. 4a we find, assuming t_0 to be arbitrarily equal to 1 hour, that if species 1 takes 48 hours to cross a given distance, species 2 will take slightly more than half that time. Thus, despite a lower mobility larger species can move more rapidly through the soil because the effect of constricting pore necks is to reduce the effective tortuosity. Because of this effect and the influence of habitat topology on refuge space, microbial dynamics measured in a medium which differs in structure from that found in the field, are likely to bear little resemblance to the actual dynamics.

The values of ϕ were measured for both soils in Fig. 4a, b and were found to be statistically indistinguishable. This is because although the relative decrease in tortuosity experienced by the larger species is greater in soil A compared with soil B (aiding diffusive flow in A relative to B), this is offset by the fact that the relative increase in homogeneity is more in soil A than in soil B (abetting diffusive flow in A relative to B). In both soils, structure bestows the advantage of greatest mobility on an otherwise more slowly moving, larger predator.

It is clear from the order of magnitude considerations presented above that to understand the dynamics of the soil microbial population it is necessary to have a quantitative framework which encapsulates soil structure. Since the rate of nutrient cycling depends on the spatio-temporal dynamics of microbes, by corollary this will not be understood without reference to structure.

CONCLUSIONS

The theory of fractal geometry is presented with reference to transport processes in soil, fungal foraging and temporal dynamics of microbes. It is shown

that when structure is properly taken into account, the classical diffusion equation is inappropriate for treating diffusive flow in soil. An appropriate theory is presented in which the fracton dimension is defined and represents a complementary measure of structure to the fractal dimension necessary for the complete description of soil structure appropriate to the problem of diffusion. Fractal geometry may also be used to probe the mechanisms of resource acquisition by a species of soil fungus. From such an analysis, it is conjectured that the processes leading to branching and determining hyphal mass distribution are independent. Finally, the implication of soil structure for the spatial and temporal dynamics of soil-borne microbes is explored. Examples are provided which suggest that an understanding of microbial processes including nutrient cycling, must include soil structure consideration. We propose that fractal geometry may provide a valuable theoretical framework to unify many aspects of the complex interrelationships between the physical, chemical and biological elements which constitute the soil ecosystem.

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Some population sizes and effects of the Enchytraeidae (Oligochaeta) on soil structure in a selection of Scottish soils

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ABSTRACT

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This paper is a summary of the results obtained from an investigation of the occurrence of Enchytraeidae in several Scottish soils. Population counts from two sites, one consisting of a rotation of eight crops each grown on plots at four different pH levels and the other consisting of a mixed pine and larch forest, show the highest numbers of enchytraeids at low soil pH levels. Soil thin sections from five sites show that at low pH the main effects of enchytraeids on soil structure are (1) the production of a large number of small granules (faecal pellets) which later fuse to form a soil matrix, and (2) their deposition in channels, other pores and old roots. At higher pH levels enchytraeids consume earthworm aggregates and replace them with their small faecal pellets and they form new pores due to their burrowing activities.

INTRODUCTION

Enchytraeids are small, white worms belonging to the Annelida, Oligochaeta. They constitute a high proportion of the total faunal biomass in many northern ecosystems, especially tundra, moorland and coniferous forest. They are particularly abundant in soils of low pH values and high organic matter content. Peachey (1963) recorded up to 290,000 m⁻² in Pennine moorland in northern England. Several authors have studied soil thin sections and report significant amounts of enchytraeid faecal material (Kubišna, 1955; Zachariae, 1963; Bal, 1973) but their effect in agricultural soils has been gener-

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TABLE I
Summary of the site characteristics

	Site 1	Site 2	Site 3	Site 4	Site 5
Site	Glentamar	Craigendarroch	Birkhall	Birkhall	Craibstone
Map reference	NO 482/923	NO 362/965	NO 351/941	NO 343/942	NJ 870/107
Vegetation	Ancient Scots Pine forest	Old oak and silver birch forest	Mixed pine and larch forest	Felled mixed forest of larch Scots Pine and Douglas Fir	Agricultural rotation (8 crops)
Soil type ^a	Podzol	Cambisol	Podzol	Cambisol	Podzol
pH	3.5-4.0	3.9-4.7	3.8-4.6	5.4	4.5-7.5
Elevation (m)	300	265	217	315	100
Rainfall ^b (mm)	880	907	880	880	816
Temperature ^c (°C)	6-7	6	6	6	8

^aFreely drained.
^bAnnual.
^cMean annual.

ally overlooked except for the work by Didden (1990). In this study a range of soils were examined including natural, semi-natural and cultivated areas.

SITE DESCRIPTIONS AND TECHNIQUES

Five sites in northeastern Scotland have been investigated. The sites (see Table 1) include very old, natural Scots Pine forest, natural scrubby oak and silver birch woodland, semi-natural mixed pine and larch forest, felled mixed forest of larch, Scots Pine and Douglas Fir and two-year-old pasture from an experimental site where a rotation of eight crops is grown on a series of pH plots (from pH 4.5 to 7.5). Samples were collected for the determination of pH, estimation of the enchytraeid population and the preparation of thin sections. The mixed pine and larch site was sampled once in late summer 1988 (C. Dersigny, pers. commun., 1988). Bulk samples were collected from the agricultural site every 4 months from September 1989 to September 1990, taking two samples from each plot, each divided into three depths. pH values were measured using an effective concentration of 0.01M CaCl_2 . For statistical analysis results were combined across all crops to test for differences between pH levels. The enchytraeids were extracted from the soil using the modified Baermann funnel method (O'Connor, 1962) and were identified according to the key of Nielsen and Christensen (1959, 1961, 1963). Thin sections of the top 10 cm of soil were prepared from all sites according to FitzPatrick (1984).

RESULTS AND DISCUSSION

In most natural and semi-natural mull soils enchytraeid populations vary between 1000 and 100,000 m^{-2} . The population count from the mixed pine and larch forest was 93,300 m^{-2} (C. Dersigny, pers. commun., 1988) agreeing with the work of other authors. In arable soils, however, populations are usually quoted as falling between 2000 and 10,000 m^{-2} but the population counts from the agricultural site in this study reached a maximum of 79,000 m^{-2} in the two-year-old pasture at pH 4.5, with an average over one year of 46,500 m^{-2} at pH 4.5, 15,600 m^{-2} at pH 5.5, 10,100 m^{-2} at pH 6.5 and 5300 m^{-2} at pH 7.5. This is shown graphically in Fig. 1.

The identification of enchytraeids shows that at low pH (4.5) the species are all small, dominantly being *Marionina argentea* (Michaelsen) 1889. At low pH in a highly organic soil *Cognettia sphagnetorum* (Vejdovsky) 1877 is usually most abundant. At higher pH (5.5 upwards) they are much larger, the majority being species of *Fridericia*.

In the thin sections from all sites enchytraeid faecal material is prominent. In one thin section from site 3 a cross-section through an enchytraeid worm occurs with a faecal pellet in the centre. Collembolan faecal material can be

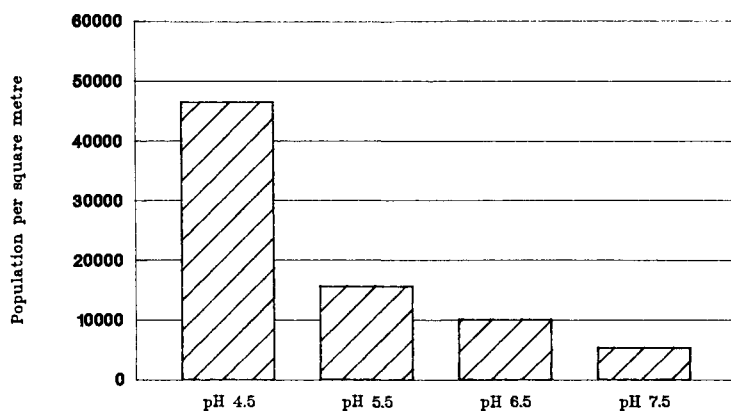


Fig. 1. Enchytraeid population size at different pH levels. Average population measured from the pH plots on the 2-year-old pasture of site 5 between September 1989 and September 1990.

confused with that of enchytraeids but they are low in number in acidic soils and could not have formed the large amounts of faecal material seen, on the other hand enchytraeid faecal pellets can be easily distinguished from those of various mites and larvae which are much larger. Enchytraeid pellets are dominantly oval in shape, with maximum diameters of between 50 and 70 μm or between 100 and 200 μm , subangular to rounded edges with abrupt boundaries, dark to light brown in colour and composed of a mixture of mineral grains, organic matter and rare fungal hyphae (see Fig. 2). Their exact colour and composition reflects that of the surrounding soil.

The frequency and distribution of the enchytraeid faecal material varied between the sites and it was found that the sites could be divided into two groups. The first had a soil pH of less than 4.8 and the second had a pH of more than 5.4. At the lower pH, three main effects on soil structure were observed. The enchytraeids produce relatively stable, small faecal pellets ranging from very frequent (10–15% of the surface area in thin section) to very abundant (25–50%). These pellets (1) dominantly occur randomly throughout the soil but (2) also commonly occur in clusters filling in channels, other pores and dead roots, and (3) with ageing they fuse together to form a soil matrix. At higher pH the presence of earthworms is known to significantly decrease the enchytraeid population due to competition (Górny, 1984). At this pH the enchytraeid faecal pellets are seen to be less abundant, only up to very frequent (10–15%), but are of the larger size. They appear identical to the smaller pellets except for their size and thus it is suggested that they may be formed by the larger enchytraeid worms (*Fridericia* sp.) which are predominant at higher soil pH. These species are also known to form burrows in the soil (Standen, 1984). In addition, it seems that at higher pH levels the enchytraeids ingest earthworm vermiforms replacing them by their much smaller faecal pellets.

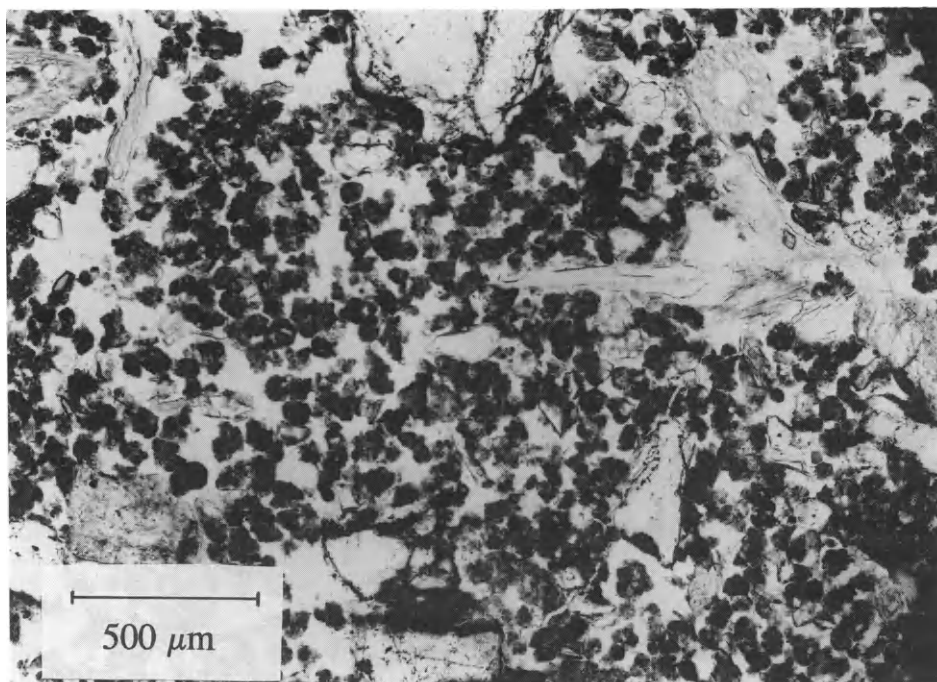


Fig. 2. Thin section of soil (0–10 cm) under the 2-year-old pasture of site 5 showing an abundance of enchytraeid faecal pellets.

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The role of roots, fungi and bacteria on clay particle organization. An experimental approach

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Experimental studies were conducted to investigate the role of different living organisms (roots, fungi, bacteria) on clay microfabric. For this purpose various microorganisms (bacteria, fungi) and roots were allowed to grow on kaolinite or montmorillonite pastes under controlled hydric conditions (-0.01 to -10^2 MPa). Microstructures were studied with a cold-stage SEM, that allowed the preservation of the organizations that characterized the wet states. With fungi, three main effects were observed in moist conditions: orientation of clay particles around the cells; secretion of extracellular polysaccharides that induced local binding of clay particles, and a general packing effect by hyphae. These effects lead to a new microstructure, in the immediate surrounding of the cell, designated as a microenvironment. A modified microstructure was recognized with all species, the size depending on the size of organism. With bacteria, polysaccharide-mediated aggregation was predominant. With grass roots, modifications of the microstructure were more complex than with fungi and occurred at a larger scale. The present results thereby confirm and complete the existing models on biologically mediated aggregation in soils.

INTRODUCTION

Monnier (1965) has emphasized the sudden increase in structural stability resulting from the incorporation of green manure to soils. This phenomenon was related to an increase in microorganism numbers and activity.

The observation of natural soil aggregates enabled demonstration of the entanglement of soil particles or aggregates by filamentous fungi or roots (Tisdall and Oades, 1982; Lynch and Bragg, 1985). However, it was difficult to separate the specific role of each organism and the basic phenomena in-

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TABLE I

Main characteristics of the experimental system

Mineral material	Living organisms	Conditions of the interaction
<i>Models of clay</i> – Wyoming montmorillonite (three-dimensional network of quasicrystals) – St-Austell kaolinite (rigid particles)	<i>Biological type</i> – bacteria: <i>Xanthomonas campestris</i> – fungi: filamentous hyphae: <i>Chaetomium</i> sp., <i>Aspergillus niger</i> , <i>Trichoderma viride</i> , <i>Corticium solani</i> , <i>Fusarium oxysporum</i> yeast: <i>Saccharomyces cerevisiae</i> – grass: <i>Triticum</i> , <i>Festuca rubra</i>	<i>Water stress</i> – moist state: -0.01 MPa = pF 2 – desiccation: -0.01 to -100 MPa (pF 2 to 6) – rehydration: -100 to -0.01 MPa (pF 6 to 2) <i>Duration of growth</i> from 2 days to six months <i>Conditions of growth</i> sterility or not nutrient solution (glucose + KNO_3)
<i>Other materials</i> – clay from alpine soils (microdivided micas) – micas with silt sized particles	<i>Morphology</i> shape: filamental (hyphae, roots) or spherical (bacteria, yeast) size: from $2\text{ }\mu\text{m}$ to several mm	

volved in the initiation and stabilization of aggregates. Studies were therefore conducted in the laboratory, either with the inoculation of selected microbial species (Harris et al., 1964; Aspiras et al., 1971), or with the selective inhibition of bacteria or fungi (Metzger et al., 1987). The increase of structural stability with many microorganisms, the prominent role of filamentous fungi, and the involvement of extracellular compounds such as polysaccharides were then emphasized (Robert and Chenu, 1992). These studies provided the basic results for more theoretical models in which each level of soil aggregation was ascribed to a major agent (Tisdall and Oades, 1982).

However, it is mostly the stabilization of preexisting aggregates that has been addressed, rather than the first stages of aggregation that imply changes in the disposition of soil particles (Allison, 1968). Furthermore, the arrangement of mineral particles, and especially of clay particles, is of uppermost importance in the macroscopic properties of soils (Emerson et al., 1986; Murray and Quirk, 1990). Recent studies have dealt with the microstructure of clay minerals and how it was affected by moisture regimes and organic matter (Chenu, 1989; Tessier, 1990); however, the effect of microorganisms was not specifically studied. Hence, the aims of the present paper were: (1) to characterize the fabrics that may be initiated in an homogeneous clay material during the development of living organisms of the soil microflora or by roots; (2) to compare the effects of organisms of various type, growing pattern and size; and (3) to separate the individual mechanisms that might be involved in the structuration by living organisms.

These results should allow to complete the existing models of biologically mediated aggregation (Tisdall and Oades, 1982) by considerations on the growing patterns of organisms and the interactions of elementary mechanisms acting at various levels of organization (from micron to millimetre).

Scanning electron microscopy has been shown to be a very adequate tool for studying clay microstructure since it provides enough resolution for the observation of micron scale particles. It was selected as the main method in this study. In addition, low temperature techniques allow the preservation of wet-state organizations, for clay minerals (Tessier, 1987), as well as for microorganisms and biological components (Beckett et al., 1982; Kellenberger, 1987).

MATERIALS AND METHODS

Living organisms

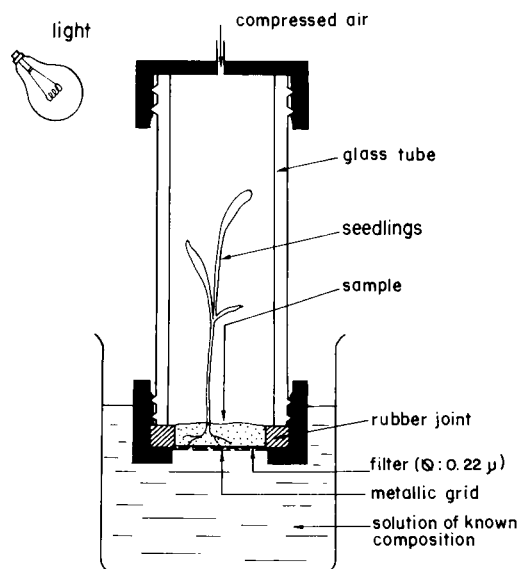
Various soil organisms were used: bacteria, fungi and young plant seedlings (Table 1). The species of fungi were selected in order to represent different shapes (yeast or filamentous fungi), sizes (3–50 μm) and modes of colonization (mycelium, colony).

Clay minerals

Two very different commercial clay minerals were selected as models for clay organizations: Wyoming montmorillonite as a model for smectites in which the quasicrystals are arranged in a continuous three-dimensional network, and St-Austell kaolinite as a model for clays with rigid particles (Tessier, 1990). St-Austell kaolinite was used in its commercial form and Wyoming montmorillonite was prepared to a calcium form as previously described by Ben Rhaïem et al. (1986). A soil clay fraction was obtained from an alpine soil (Table 1).

Experimental procedure

Concentrated suspensions of the clay were impregnated with simplified nutrient solutions: for fungi and bacteria glucose 0.1 g kg^{-1} and KNO_3 0.02 g kg^{-1} ; for seedlings KNO_3 0.01 g kg^{-1} and K_2PO_4 0.01 g kg^{-1} . The clay suspensions were then equilibrated to a matric potential of -0.1 MPa by applying an air pressure of 0.1 MPa in a filtration device adapted from Tessier and Berrier (1979) (Fig. 1). Once equilibrium had been reached (the clay pastes then had a water content of 1.0 g g^{-1} for kaolinite and 3.2 g g^{-1} for montmorillonite), the clay was inoculated on the surface or planted with the ap-



Ultrafiltration apparatus used for $pF < 3$

Fig. 1. Ultrafiltration apparatus (for $pF < 3$) used to settle the matric potential (for $\Psi \geq -0.1 \text{ MPa}$).

propriate organism. The sample was maintained under constant (-0.01 MPa= pF_2) or fluctuating matric potential ($-0.01 \rightarrow -100 \rightarrow -0.01$ MPa= $pF_2 \rightarrow 6 \rightarrow 2$) in the same filtration device; however, -100 MPa or pF_6 was obtained by air drying. Temperature was kept constant at 20°C . Seedlings were illuminated.

Aseptic conditions were obtained in several steps, first the filtration device was autoclaved, second the clay suspensions were sterilized at 100°C for 5 min in an autoclave. This treatment suppressed most clay contamination (J. Schmit, pers. commun.). By X-ray diffraction measurements and scanning electron microscopy it was checked that sterilization and impregnation with nutrient solutions did not alter the clay organization. Then the microbes or seedlings were aseptically inoculated to the clay paste and, once the device was set up, contamination was prevented by $0.22\ \mu\text{m}$ filters separating the samples from both the incoming air and the nutrient solution (Fig. 1). For seedlings, contamination was widely reduced with a treatment in alcohol (5 min) followed by a short impregnation with NaOCl (30 s). This overall procedure was sufficient to prevent competition between contaminants and introduced microorganisms which were also selected as being very competitive species.

Observation conditions

Scanning Electron Microscopy (SEM) observations of the modified or reference clay samples were performed with a specific method (cryoSEM) designed to preserve the wet-state organizations. A fraction of several mm^3 of the wet sample was frozen at -146°C in freon cooled by liquid nitrogen; then introduced in a special chamber at -40°C adapted to the SEM where it was fractured, superficially lyophilized and coated with gold. After introduction in the Jeol 35 SEM column itself, the sample was observed at -100°C . This procedure prevents the samples from drying in the air, as necessary for conventional SEM, by fixing the structures that occur in the wet state. The absence of major artefacts was confirmed on pure clays or clay-organic matter associations, by other studies of the microstructure (Tessier, 1987; Chenu, 1993a). On the other hand for clay-fungus and clay-organic matter mixtures, we compared the fabric of samples prepared either by a critical point drying method (with acetone and CO_2) or with the cryoSEM method. Very similar clay fabrics, microbes and polysaccharides shapes were obtained in both cases, as also stated by Beckett et al. (1982). However, the information is more accurate with the cryoSEM method, both in the details of the clay-cell wall contact than for the overall phenomena, and the cryoSEM method is much more convenient than the critical point drying one.

RESULTS

At -0.01 MPa, extensive colonization of the clay pastes by the microbes or seedling roots was observed within a few days.

Effects of fungi

With kaolinite, from the early stages of growth (three days) at -0.01 MPa, a particular area of $1-5\text{ }\mu\text{m}$ thickness, consisting of oriented kaolinite particles was observed around the hyphae (Fig. 2a). In detailed pictures of the wall-clay interface (Fig. 2d, e) it is easier to distinguish the loose contact between the cell wall and the clay particles, and the orientation of the clay particles parallel to the wall, along with an apparent increase in the clay porosity. Many interconnected strands of about $0.1-1\text{ }\mu\text{m}$ length and $0.01\text{ }\mu\text{m}$ thickness could be observed between the clay particles. Many fungi are known to produce extracellular polysaccharides (Barretto-Bertger and Gorin, 1983; Fleet and Phaff, 1981), and the observed strands had a typical morphology of microbial extracellular polysaccharides as previously observed with the cryoSEM (Chenu, 1989). The networks of fibers observed in the present experiment were therefore assumed to be the extracellular polysaccharide of the fungi. This secretion became more abundant as the culture aged. In an older culture (one week) the clay in the environment of the hyphae was filled with polysaccharides (Fig. 2e, f) and some microfissures were also observed in the clay at -0.01 MPa (Fig. 2c).

After a desiccation-rehydration cycle ($-0.01 \rightarrow -100 \rightarrow -0.01$ MPa), the fissures increased in size and number around this microenvironment (Fig. 3a), and lead to the formation of microaggregates. The same process was observed around yeast colonies (Fig. 3b). When the samples were further brought at -0.01 MPa, the fissures were colonized by the fungi and physical entanglement of the microaggregates was observed (Fig. 3c).

With montmorillonite, the contact between the fungal wall and particles was much more intimate than with kaolinite (Fig. 2b). Frequently the quasicrystal and cell wall could not be distinguished from one another (Fig. 3d, yeast). This adhesion was the dominant feature of the close environment of either hyphae or yeast, although a parallel orientation of particles, a lower mean pore size, and a polysaccharide secretion were also observed (Fig. 2b).

Effects of bacteria

Bacteria were always very difficult to locate in clay matrices (Fig. 3e), as a result of their small size, of their adhesion to clay particles and of the coating of the cells with extracellular polysaccharides (Fig. 3e). The major effects of bacteria on the clay fabric (kaolinite or montmorillonite) were the adhesion

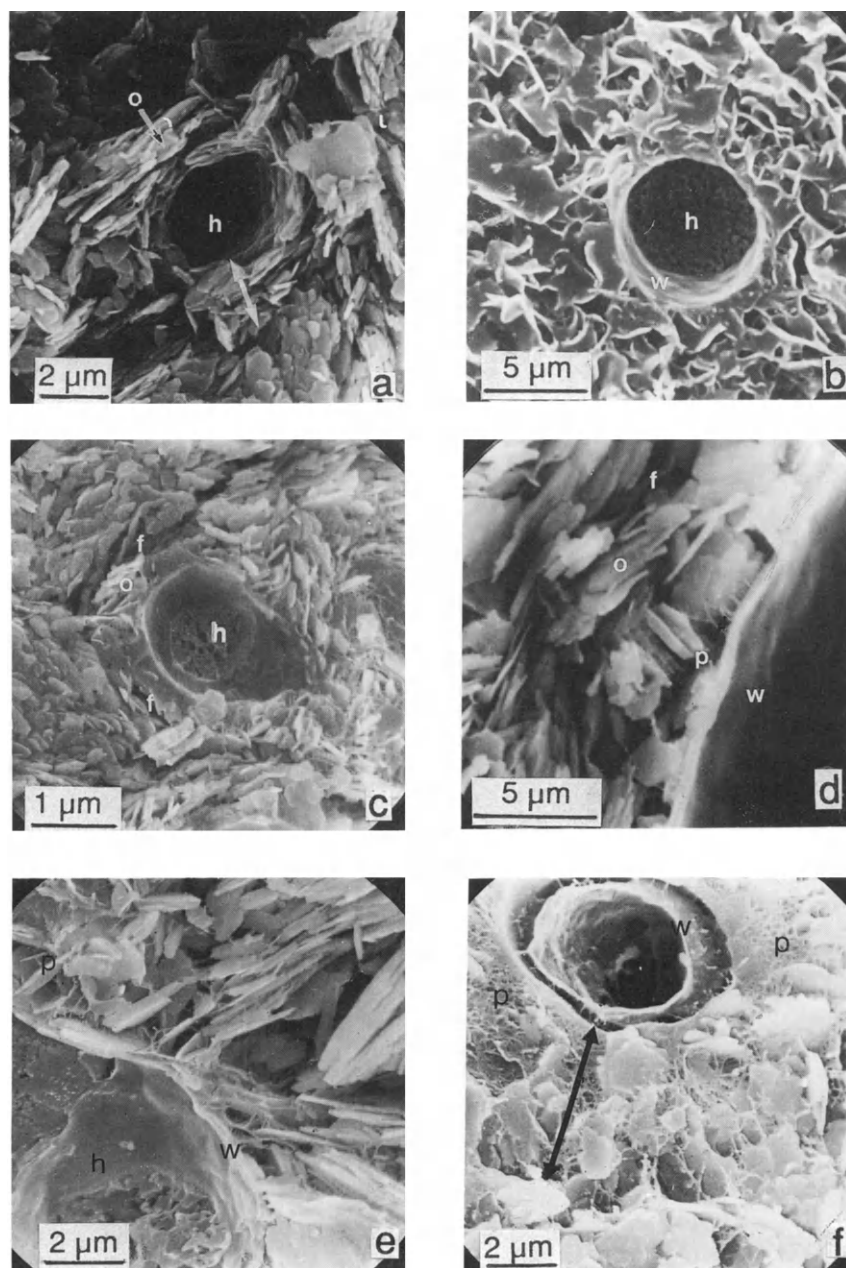


Fig. 2. Microstructures induced by filamentous fungi in kaolinite at -0.01 MPa. CryoSEM observations. (a,b) *Chaetomium* sp., early stage of growth. (c,f) *Chaetomium* sp., older culture. (d) *Corticium solani*, early stage of growth. (e) *Fusarium oxisporum*, early stage of growth. Key for Figs. 1–5: *h*=hyphae, *w*=cell wall; *o*=oriented clay particles; *f*=fissure; *p*=polysaccharide; $\leftrightarrow$ =microenvironment.

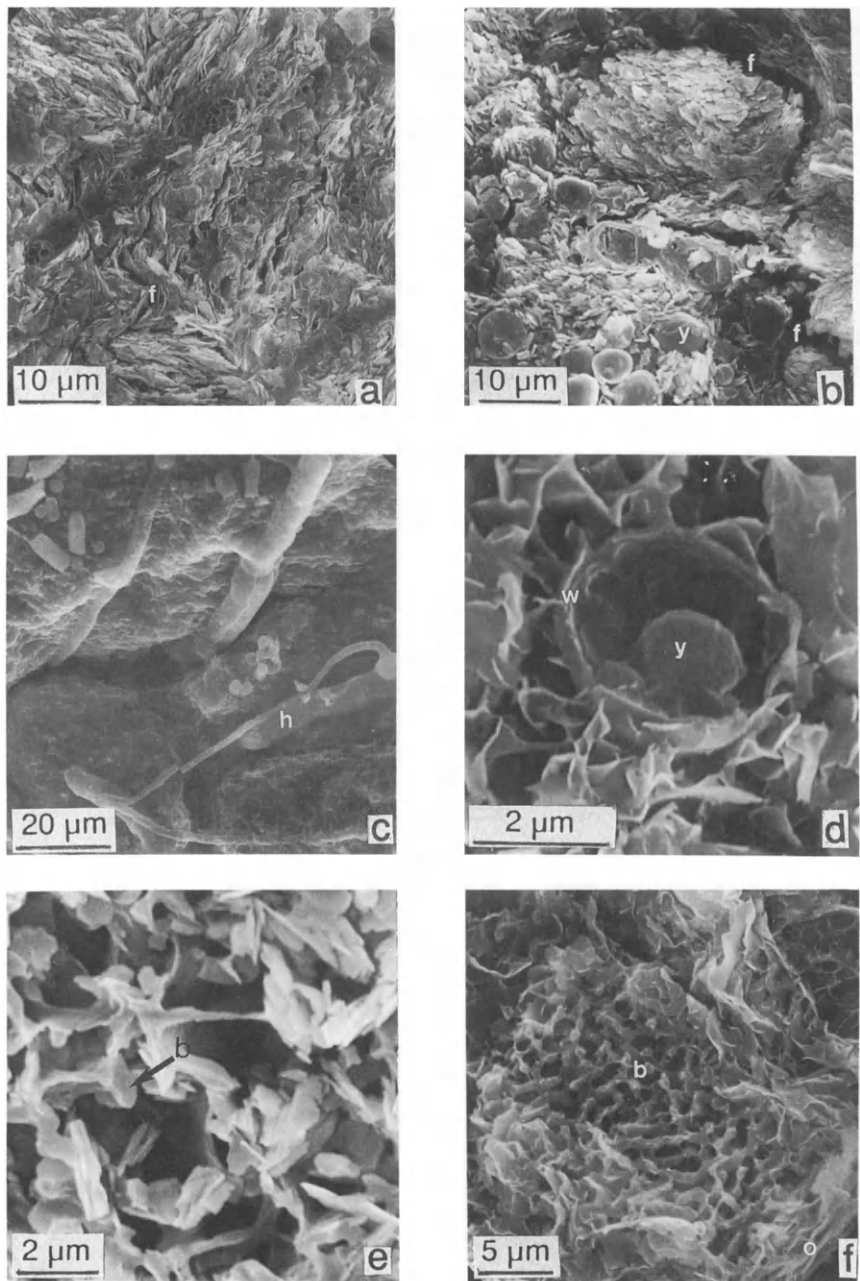


Fig. 3. Microstructures induced by bacteria or fungi in clays. CryoSEM observations. (a) *Trichoderma viridis* in kaolinite rehydrated from -10 to -0.01 MPa. (b) Microaggregation of kaolinite close to a yeast colony, after rehydration from -10 to -0.01 MPa (*Saccharomyces cerevisiae*). (c) Mycelium colonizing a fissure in rehydrated Ca-montmorillonite (from -10 to -0.01 MPa, *Chaetomium* sp.). (d) Yeast cell with ascospore in Ca-montmorillonite (-0.01 MPa, *Saccharomyces cerevisiae*). (e) *Xanthomonas campestris* adhering to Ca-kaolinite particles (-0.001 MPa). (f) Bacterial colony in Ca-montmorillonite at -0.01 MPa. For key see Fig. 2; *b*=bacteria, *y*=yeast.

of the clay platelets to the cell and a strong polysaccharide secretion that penetrated the surrounding clay pores (Fig. 3e). Such adhesion of clay particles to bacteria explains the formation of microaggregates which can be seen by ultrathin section and transmission electron microscopy. In the case of *Xanthomonas campestris* (Fig. 3e), the extracellular polysaccharide was xanthan (Robert and Schmit, 1982). Orientation of clay particles was only observed with large bacterial colonies and was not very pronounced (Fig. 3f).

Effects of roots

Due to difficulties in collecting samples, we have only characterized the activity of the tip of young roots (up to seven days) with root hair. However the root apex is physiologically very active and displays a specific behaviour: abundant secretion of mucilages, strong constraints due to growth and nutrient adsorption. The particularly active part of the root in soil fabric is well documented (Foster and Rovira, 1976; Habib, 1988).

Around the root hairs, modifications of the clay in a microenvironment of about 10 μm in diameter were clearly visible (Fig. 4a, b), and they were similar to what was observed with fungi. With the whole root, microstructural effects on kaolinite were much stronger than those with hyphae (Fig. 3c). The reoriented area was larger (20–50 μm), more irregular in shape and very rich in root mucigel (Fig. 4e). A greater number of microfissures occurred in continuously moist conditions and only a few clusters of crystallites remained in close contact to the cell wall of the root than with the hyphae (Fig. 4d).

Such fissures, in the absence of any desiccation event, were also observed in montmorillonite. The cracks and the separation area around the root were filled with polysaccharides (Fig. 4c, d in kaolinite). After a desiccation–rehydration event, the concentric fissures around the root were increased in number as well as in size, and fissures were also observed to develop from root hair.

With mineral particles of greater size (such as silt- or sand-sized particles of phlogopite or mica) the effects of the roots on the microstructure of the mineral were less important as the surface area of the interface between living organisms and mineral particles was decreased. It was observed, however (Fig. 4f), that the smallest particles tended to be located preferentially near the root, as the result of a granulometric sorting.

Under non-sterile conditions, microbial penetration occurred first from the root axis (Fig. 5a). The root mucigel acted as a nutrient medium for bacterial populations (Fig. 5b). The specific action of this microflora on clay fabric was then combined with that of the root itself. Direct clay–root interaction appeared to take place only in a few microns beyond the root cap, whereas over the pilifer zone direct clay–microorganisms interactions were predominant. The overall action of the root, together with root hair and associated

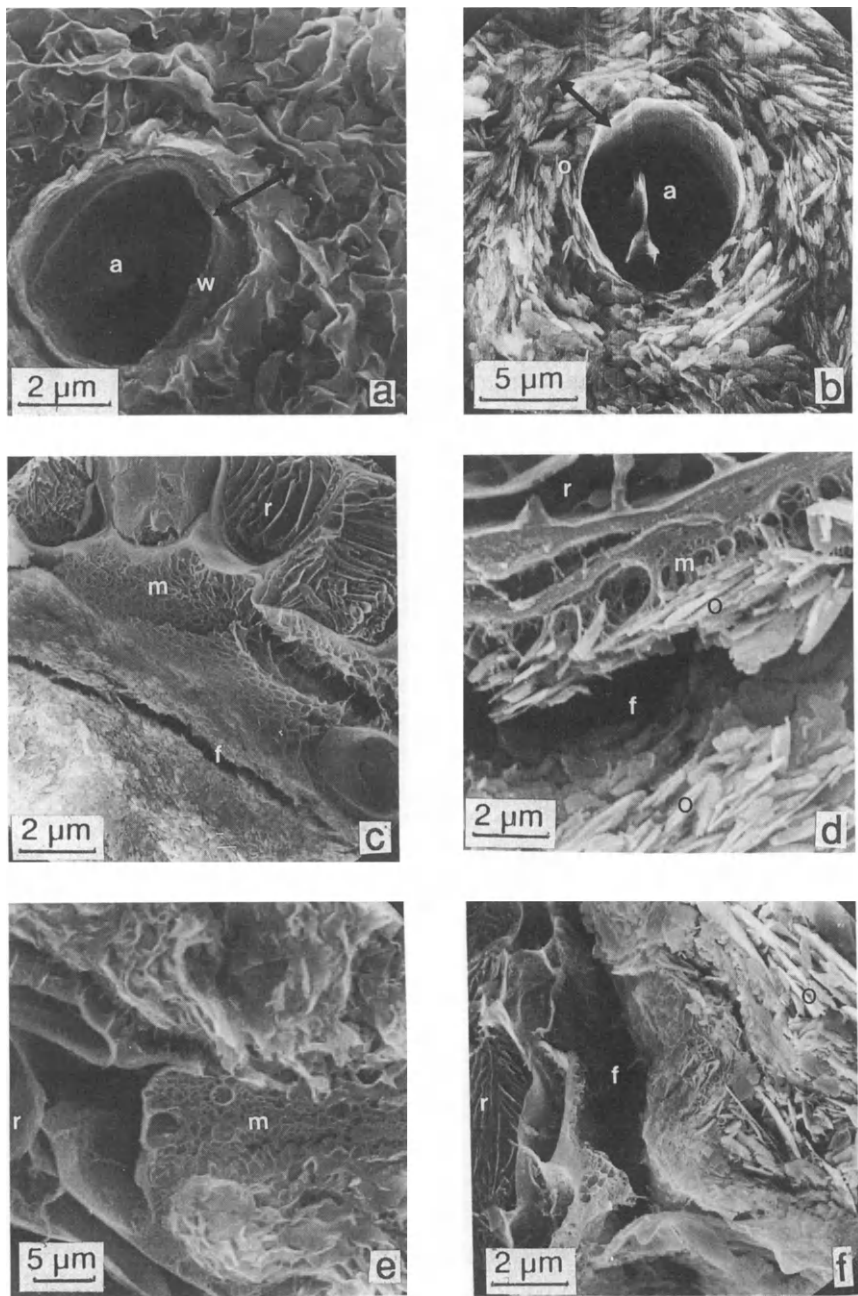


Fig. 4. Microstructures induced by tip roots in clays. CryoSEM observations. (a) Absorbent hair of *Festuca rubra* in Ca-montmorillonite (-0.01 MPa). (b) Absorbent hair of *Festuca rubra* in kaolinite (-0.01 MPa). (c) Young wheat root in kaolinite (-0.01 MPa); $\leftrightarrow$ = root microenvironment: well oriented kaolinite, abundant mucigel; (d) Detail of wheat root-kaolinite interface (-0.01 MPa). (e) Wheat root-montmorillonite interface (-0.01 MPa), m = mucigel, a = absorbent hair, r = root cell. For key see Fig. 2.

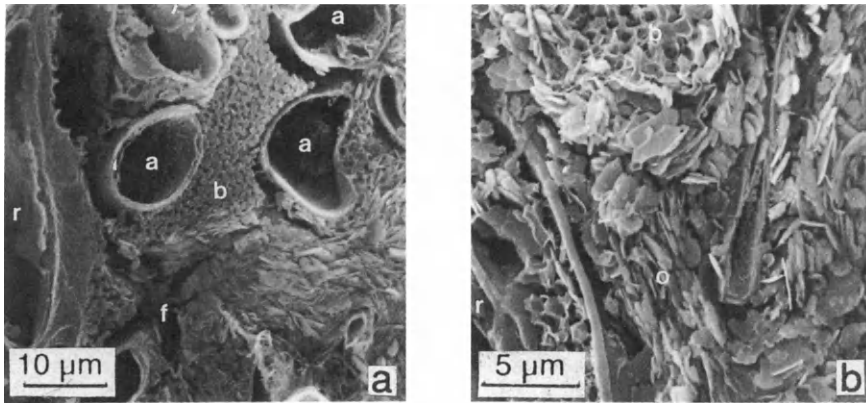


Fig. 5. Microstructures induced by *Festuca rubra* rhizosphere in kaolinite at -0.01 MPa. CryoSEM observations. *o*=oriented particles; *a*=root hair; *b*=bacterial colonies. For key see Fig. 2.

bacteria and fungi lead to the modification of a zone of about 1 mm in diameter, that corresponded to the rhizosphere. This large volume had a looser structure than at the scale of the root or root hair microenvironments.

DISCUSSION

Our experimentations supplied information on the succession of basic phenomena involved in initiating and stabilizing microaggregates by various living organisms:

(1) Mechanical effects were due to penetration of roots or hyphae or to the suction resulting from adsorption of water by the organisms, which amount to physical constraints on clay. Such mechanisms promoted a reorganization of the clay, characterized by oriented and compacted clay particles. The size of the modified area depended on the size of the living organisms: $5\text{--}20\text{ }\mu\text{m}$ for a cell (fungi, root hair) or a bacterial colony, $50\text{--}200\text{ }\mu\text{m}$ for a root. In the case of individual bacterial cells, because of the size ratio of about 1 between cells and clay particles, this structural effect did not exist. It would, however, probably occur with smaller soil particles, which are more generally found in soils.

(2) A polysaccharide effect was due to the extracellular secretion of polysaccharides by many living organisms, bacteria as well as fungi or roots. The polysaccharides penetrated and impregnated the surrounding mineral phase, to a distance of less than $1\text{ }\mu\text{m}$ for bacteria (Fig. 3e) and up to $50\text{ }\mu\text{m}$ for root tips (Fig. 4e). The actual polysaccharide concentrations in this volume are rather difficult to estimate and are probably very high, much higher than the average polysaccharide content in the bulk material. The presence and abun-

dance of polysaccharides in the vicinity of microorganisms and roots were also recognized in soil samples by several authors using transmission electron microscopy (Guckert et al., 1975; Foster and Rovira, 1976; Kilbertus et al., 1979; Foster, 1988). Polysaccharides seem to be widely present as an interface between soil biota and the surrounding minerals. Furthermore, as many polysaccharides have a tendency to adsorb onto clay minerals (Theng, 1979), it is a clay-polysaccharide association that is likely to constitute the microenvironment of soil microbes, as clearly observed in the present study. The size of this microenvironment is expected to depend also on abundance of the polysaccharidic secretion and molecular characteristics (molecular weight, charge, viscosity) that are likely to influence the reaction with clay minerals and diffusion in the solid matrix.

The physical properties of clay-polysaccharide associations are quite different from those of pure clays. The water retention of clays is increased and shrinkage and swelling patterns are thus modified (Chenu, 1989, 1992b). With some extracellular polysaccharides, such as scleroglucan from fungi or xanthan from bacteria, bridging of the clay particles is achieved, enabling stronger cohesion and increased water stability of the clay material (Chenu and Guérif, 1991; Chenu, 1993b). With root mucigel (Habib et al., 1991) or its main constituent, e.g., polygalacturonic acid (Chenu, unpublished) a very strong interaction that lead to microaggregation was observed.

Considering that experimentally prepared clay-polysaccharides associations are good models for the microenvironment of microbes, it is deduced that in the present experiments, exuded polysaccharides stabilized the structure of the mineral soil fraction. Polysaccharides participated in the formation of a microenvironment around each living organism (5–50 μm) with a specific microstructure and physical properties, that tended to isolate the cell from the non-perturbed bulk clay.

Microorganisms and roots induced microfissures in the clay, even in continuously moist conditions and in poorly reactive clays such as kaolinite. It is likely that the combination of elementary effects, i.e., mechanical stress and polysaccharide secretion resulted in physical properties in the bulk clay and in the microenvironment which were significantly different for the two to become separated by fissures. In the case of roots, these microfissures were, in a second phase, often filled with polysaccharide, probably maintaining a root-clay continuity.

(3) Steric effects also took place as the modified microenvironments of the cells or roots themselves represented discontinuities in the mineral phase. It was demonstrated with roots that fissures often develop from the discontinuities they form, either tangentially or around the border of the modified zone (Tri and Monnier, 1973). In the present study steric effects were generally due to fungi but were enhanced by drying and rewetting events. This interaction between a physical process and a local and drastic modification due to

an organism, in an initially homogeneous material, lead earlier to well-individualized microaggregates (Dorioz and Robert, 1982). Thus a microenvironment may lead to a microaggregate. Bacteria may build up microaggregates as follows: adhesion of cells to clay particles or of clay particles to cells, strengthening of the adhesion by polysaccharides and further by drying–rewetting cycles.

(4) Packing effects were outstanding with mycelia networks growing in the fissures and entangling the mass of clay. This physical entanglement also took place with root hair and root axis, was observed but at a different scale when all of the root system was included. It tended to create a macroenvironment (50–200 μm) around the root.

The elementary effects distinguished here were common to all the living organisms studied. The experimental approach of the effects of soil biota on clay fabrics, confirmed the hierarchical view of soil aggregation proposed by Tisdall and Oades (1982): bacteria were responsible for micron scale microenvironments, fungi for 5–20 μm ones. Roots and root hairs induced organization of the solid matrix on the scale of a macroenvironment (20–200 μm) and filamentous organisms (hyphae, roots + root hair) exerted, because of their growth, a large-scale packing effect (50–1000 μm). The outstanding effect of the rhizosphere on soil structure can be related to the rhizosphere as being the privileged site for growth for a wide range of microorganisms of various sizes, each of them organizing the material at its own scale. In soils, the overall biological activity results in stable macroaggregates > 200 μm (Elliott, 1986).

The present study allowed us to modulate the above model as regard to the size factor (size ratio between living organism and mineral particles), the way of growth of organisms and the nature of the clay minerals.

Changes in particles organization were more apparent in clays with rigid particles, such as kaolinite, and in microdivided primary minerals such as micro-micas. With montmorillonite, adhesion of the quasicrystals to cells and polysaccharides was the main feature. With larger size particles (silt or sands), the only observed change in structure was the packing, at a large scale by hyphae or roots (Dorioz and Robert, 1987).

The extent of mechanical effects increased with the size of organisms and decreased with the size of mineral particles. Bacterial colonies were the smallest units to develop stresses upon growth that could reorientate clay particles. On the other hand, we assume that only roots may reorientate silt- or sand-sized particles. Orientate clay particles at the surface of bacteria are quite a common feature in transmission electron microscopic observations of soils (Kilbertus et al., 1979; Foster, 1988). Such organization was not observed here, probably due to the too large particle size of the kaolinite and montmorillonite used. Growth of bacteria in the clay matrices under moist conditions, as performed in the present experiments, allowed contact between the cell

and clay particles through adhesion and secretion of extracellular polysaccharides. It is hypothesized that drying–rewetting events would achieve closer contact and reorientation of the clay particles along the bacterial cell.

CONCLUSIONS

The outstanding role of polysaccharides was confirmed in this study, but the influence of living organisms was more than a strong secretion of polysaccharides in their environment and we have emphasized the importance of suction and growth of living organisms on fabric elaboration. Living organisms were shown able to induce a structure in a mineral matrix under conditions in which physical mechanisms prove ineffective: poorly reactive clay minerals (with rigid particles), large-size particles (silts and sands) and environments with poorly contrasted water potentials.

Differentiation of the mineral phase close to the organisms is the first step in aggregation processes. It is also highly significant for the biota in physiological terms such as nutrient conditions, resistance to desiccation, and for root contact with telluric pathogens (Callot et al., 1982; Schmit and Robert, 1984; Roberson and Firestone, 1992). Knowledge of interactions between biota and soil constituents is indeed of upmost importance in the prospective of sustainable agriculture, based on a better management of organic matter and even on the management of introduced or indigenous microbial populations.

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Micro-morphological studies on clay-amended and unamended loamy sand, relating survival of introduced bacteria and soil structure

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ABSTRACT

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With the use of Cryo Scanning Electron Microscopy (SEM performed at low temperatures) the microstructure of clay- (bentonite and kaolinite) amended and unamended loamy sand was studied. A wide range of pore sizes could be recognized in all three treatments, but pore size distributions clearly differed between the soil types. The bentonite clay was present as a very porous matrix, containing pores with an average maximum length of $\sim 3 \mu\text{m}$, and an average minimum width of $\sim 2 \mu\text{m}$. Pores of similar sizes were also present in kaolinite- and unamended loamy sand, but they were much less frequent. Previous results, suggesting that the protective effects of bentonite clay on the survival of introduced bacteria in soil could be caused by an increase in the number of pores with an equivalent neck diameter $< 6 \mu\text{m}$, were confirmed.

INTRODUCTION

Soil structure and texture have a large influence on the population dynamics, ecology and activity of soil microorganisms (Stotzky, 1986). For example, decomposition rates of organic matter are lower in fine-textured clayey soils, than in coarse-textured, sandy soils (Van Veen and Kuikman, 1990). Van Elsas et al. (1986) found that survival of introduced bacteria was enhanced in a silt loam as opposed to a loamy sand. Heijnen et al. (1988) found that the survival levels of *Rhizobium* introduced into loamy sand could be increased by the addition of bentonite, consisting almost entirely of smectite,

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and to a less extent by the addition of kaolinite. The increased survival could be explained by the presence of many pores with equivalent diameters of $< 6 \mu\text{m}$ in bentonite-amended soil as opposed to unamended loamy sand, which were called protective micro-habitats. Bacteria situated inside these micro-habitats are protected from predation by protozoa (Heijnen and Van Veen, 1991).

The pore size distribution of a soil can be determined in different ways (Lawrence, 1977). Examples are the use of moisture characteristic curves (Heijnen and Van Veen, 1991), or the mercury intrusion method (Wu et al., 1990). Both methods are based on a cylindrical pore model (Lawrence, 1977), but since pores are never truly cylindrical, incorrect pore diameters are likely to be determined. However, both methods are widely used for the description of the pore size distribution in soil. A different way to study pore size distributions is by (micro-) morphology, involving light-microscopic studies of soil thin sections. If samples are thin enough to be permeable to electrons, transmission electron microscopy (TEM) is also applicable (Foster et al., 1983), allowing for much higher magnifications (2000–1,000,000, Tessier, 1991). A problem arising when using soil thin sections is that soil moisture has to be replaced by resin, thereby introducing the possibility of creating artifacts due to shrinkage of the soil or through damage to, or the solubility of, organics. An application of thin sections in soil microbiology is the staining (in thin sections) of bacteria introduced into soil (Postma and Altemüller, 1990; Tippkötter, 1990).

Since it was hypothesized in previous studies (Heijnen et al., 1988; Heijnen and Van Veen, 1991) that the protective effect of bentonite clay on rhizobial survival might be due to the formation of pores with diameters $< 6 \mu\text{m}$ (Heijnen and Van Veen, 1991), it was tried to confirm this hypothesis by visualizing these pores. Cryo Scanning Electron Microscopy (CryoSEM, Tessier and Berrier, 1979) was applied, a method previously found to preserve the organization of clay materials in their original hydrated condition (Chenu, 1989). The aim of our present work was therefore to study the microstructure and to visualize small pores ($< 6 \mu\text{m}$) in bentonite and unamended loamy sand. Since kaolinite was also found to influence rhizobial survival (Heijnen and Van Veen, 1991), kaolinite-amended loamy sand was also included in our study.

MATERIALS AND METHODS

Soil and clays

A loamy sand, kept in experimental field micro-plots for several years, was used for these studies. Some soil characteristics were given by Heijnen et al. (1992). X-ray analysis showed that the silt and clay fractions in the loamy

sand mainly consisted of quartz and feldspars. Kaolinite and illite were also present, the latter with some smectite interstratification. The loamy sand was amended with kaolinite or bentonite clay, or was left unamended. The composition of the cation exchange complex of the bentonite and kaolinite, respectively, are as follows (meq/100 g dry clay mineral): Na^+ , 55.6 and 1.5; K^+ , 1.1 and 0.5; Ca^{2+} , 27.4 and 0.6; Mg^{2+} , 6.9 and 0.8.

Soil treatments

Bentonite was added as a powder to concentrations of 0.5, 1 and 5% (mass fractions) to loamy sand. Kaolinite was only added in amounts of 10%, also as a powder. The different soil mixtures were moistened to a moisture content corresponding to that occurring at a matric potential of -10 kPa, which resulted in water contents of 32% for the 5% bentonite treatment and $\sim 18\%$ for all other remaining treatments (Heijnen and Van Veen, 1991). To determine whether the pore sizes in bentonite-amended soil were influenced by the water content, loamy sand amended with 5% bentonite was also studied at a moisture content of 18% (corresponding to a matric potential of -80 kPa).

Cryo Scanning Electron Microscopy (cryoSEM)

In contrast to Tessier and Berrier (1979), a slightly modified sample preparation technique for CryoSEM studies was applied, using a Hexland CT100 cryotrans system. Soil aggregates of approximately $2\text{--}3\text{ mm}^3$, containing 18 or 32% water depending on the treatment, were cryofixed by immersion into melting nitrogen (-210°C). At this temperature ice crystal growth is limited (Robards and Sleytr, 1985), thereby minimizing the chance of the occurrence of freezing artifacts. The sample was then transferred to the preparation chamber (-185°C) where it was fractured. Ice was partially sublimated under vacuum at temperatures of about -90°C , in order to limit ice crystal growth (Robards and Sleytr, 1985). Sample surfaces were coated with gold to provide a conductive film. Samples were observed at temperatures less than -110°C in the refrigerated column of a Philips SM525 scanning electron microscope. The number of pores and their sizes were evaluated in the different soil treatments. Pores from a three-dimensional image were traced (two-dimensionally), and maximum pore lengths and minimum pore widths were estimated using a Quantimet 970 image analysis system (Cambridge Instruments).

RESULTS

In the loamy sand, without additional clay (Fig. 1), sand grains ranging from 50 to $500\text{ }\mu\text{m}$ and large interstitial pores ($\sim 100\text{ }\mu\text{m}$) were observed.

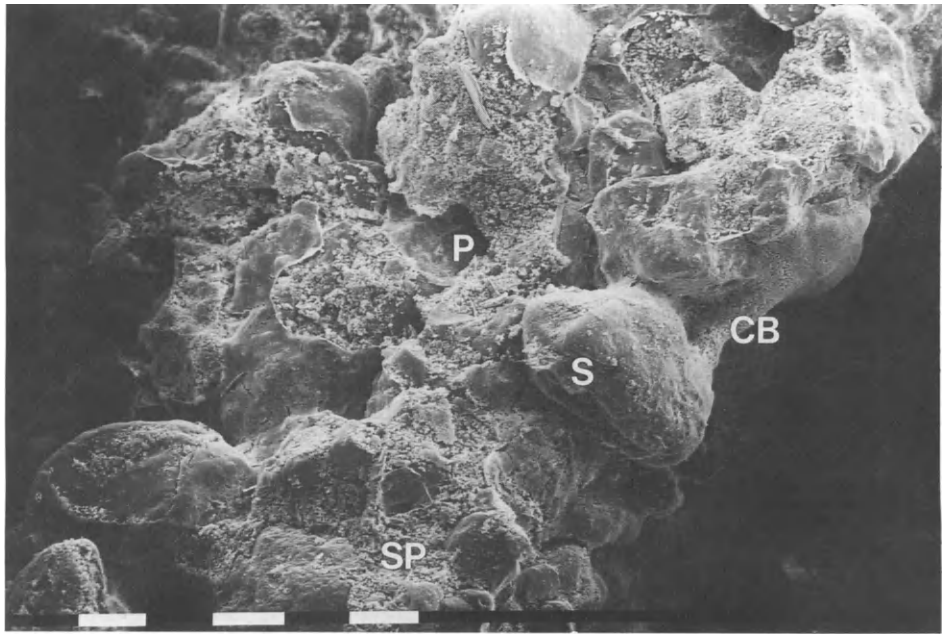


Fig. 1. Unamended loamy sand. P=pore; S=sand grain; CB=clay bridge; SP=soil plasma; white bar=0.1 mm.

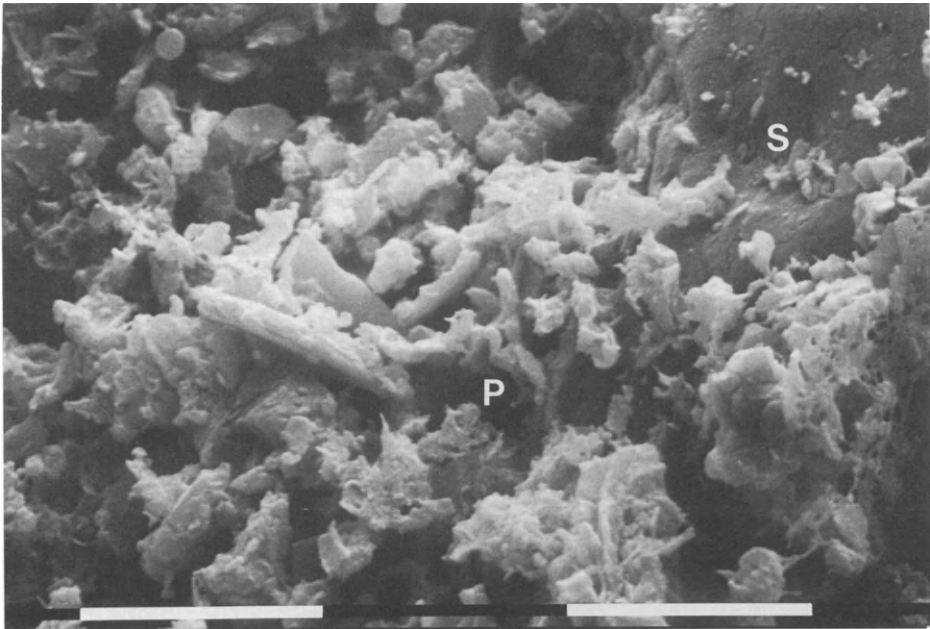


Fig. 2. Soil plasma in unamended loamy sand. P=pore; S=sand grain; white bar = 10 μm.

TABLE 1

Numbers of pores and their average length and width, including the standard deviation (SD), measured from CryoSEM micrographs

Treatment	No. of pores	Length $\pm$ SD (μm)	Width $\pm$ SD (μm)
ls	24 ± 1	5.2 ± 0.6	3.1 ± 0.2
ls + 10% k	27 ± 5	4.7 ± 1.1	1.7 ± 0.3
ls + 5% b	78 ± 5	2.7 ± 0.2	1.7 ± 0.2

Countings were performed on areas of $39.0 \times 24.5 \mu\text{m}^2$. ls = loamy sand; b = bentonite; k = kaolinite.

Some sand grains were coated with plasma (fine size fraction), whereas others were bare. The plasma consisted of clay, silt and organic matter. Clay also occurred as bridges between sand grains (Fig. 1). The plasma (Fig. 2) showed some porosity, too. In areas of $39 \times 24.5 \mu\text{m}^2$ (e.g. Fig. 2), an average of 24 pores was counted with an average maximum length of $5.2 \mu\text{m}$, and an average minimum width of $3.1 \mu\text{m}$ (Table 1). Indigenous soil clay could be recognized by its blocky appearance.

The microstructure of loamy sand amended with 10% kaolinite (Fig. 3) was completely different. Most of the sand grains were coated with plasma (mainly kaolinite), and only few bare grains remained. The large interstitial pores present had a similar size ($\sim 100 \mu\text{m}$) to those found in the unamended loamy sand. The plasma (Fig. 4) was dominated by the plate-like structure of kaolinite. The size of kaolinite plates was $2\text{--}10 \mu\text{m}$, ranging into the silt fraction. In the plasma, in areas of $39 \times 24.5 \mu\text{m}^2$ (e.g. Fig. 4), an average of 27 pores was counted with an average maximum length of $4.7 \mu\text{m}$, and an average minimum width of $1.7 \mu\text{m}$ (Table 1).

Amendment of loamy sand with 5% bentonite clay (32% moisture content) had a large effect on the soil structure (Fig. 5). Sand grains appeared to be embedded in or covered with bentonite clay, which was a dominating component of the soil plasma. Large pores ($\sim 100 \mu\text{m}$) were less frequent than in unamended and kaolinite-amended loamy sand. The bentonite formed a very porous matrix (Fig. 6), which in some places contained organic remnants (not shown). The pores had a polygonal shape with thin wrinkled walls, resembling a honeycomb structure (Fig. 7). In areas of $39 \times 24.5 \mu\text{m}^2$ (e.g. Fig. 7), an average of 78 pores was counted with an average maximum length of $2.7 \mu\text{m}$, and an average minimum width of $1.7 \mu\text{m}$ (Table 1). It was obvious that small pores were much more abundant (approximately three times) in loamy sand amended with 5% bentonite clay than in unamended or kaolinite-amended loamy sand (Table 1).

At a moisture content of only 18% in the 5% bentonite treatment a similar porous structure was visible as was the case with a moisture content of 32%

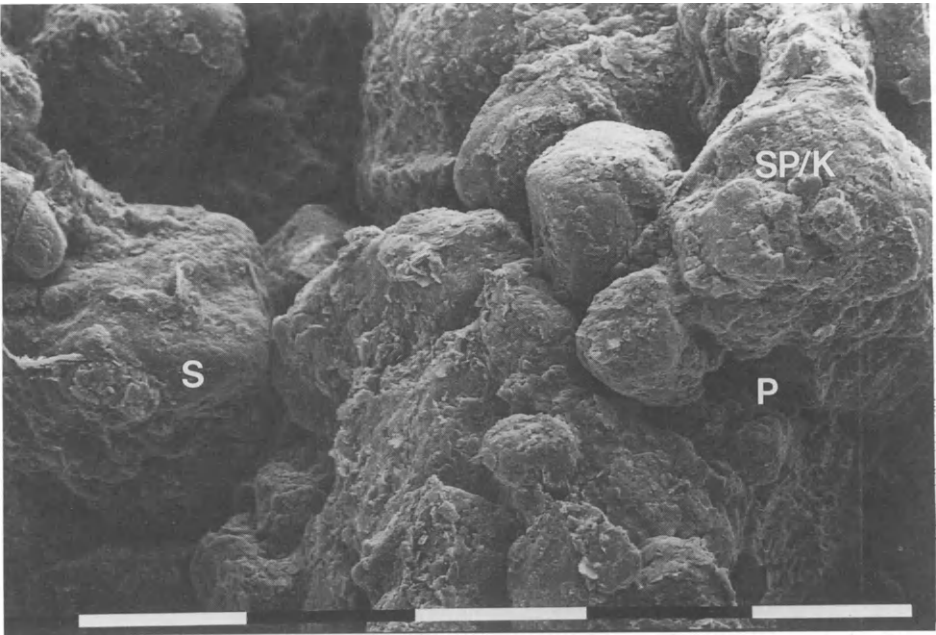


Fig. 3. Loamy sand amended with 10% kaolinite. P=pore; S=sand grain; SP=soil plasma; K=kaolinite; white bar=0.1 mm.

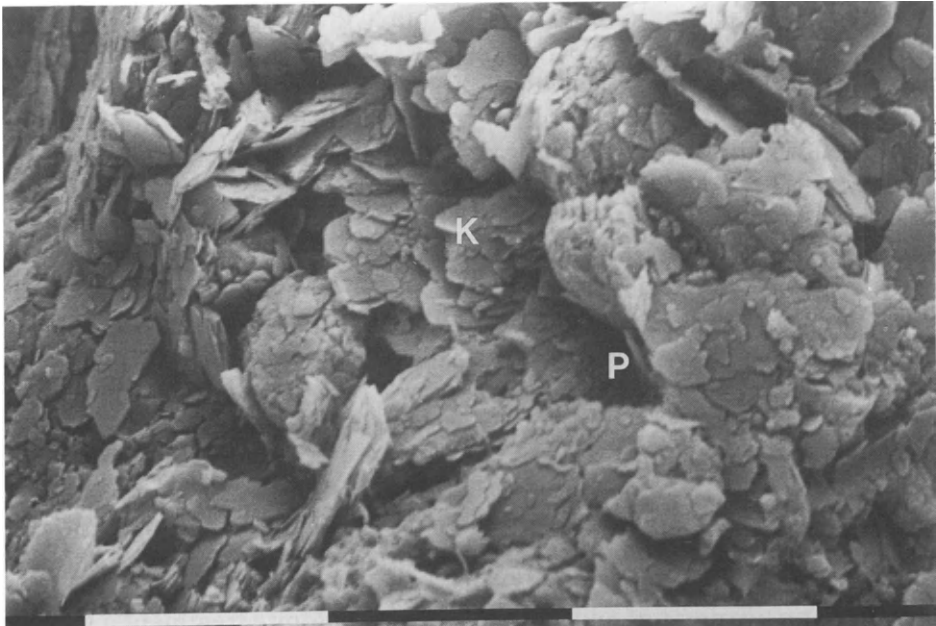


Fig. 4. Soil plasma in loamy sand amended with 10% kaolinite. P=pore; K=kaolinite; white bar=10 µm.

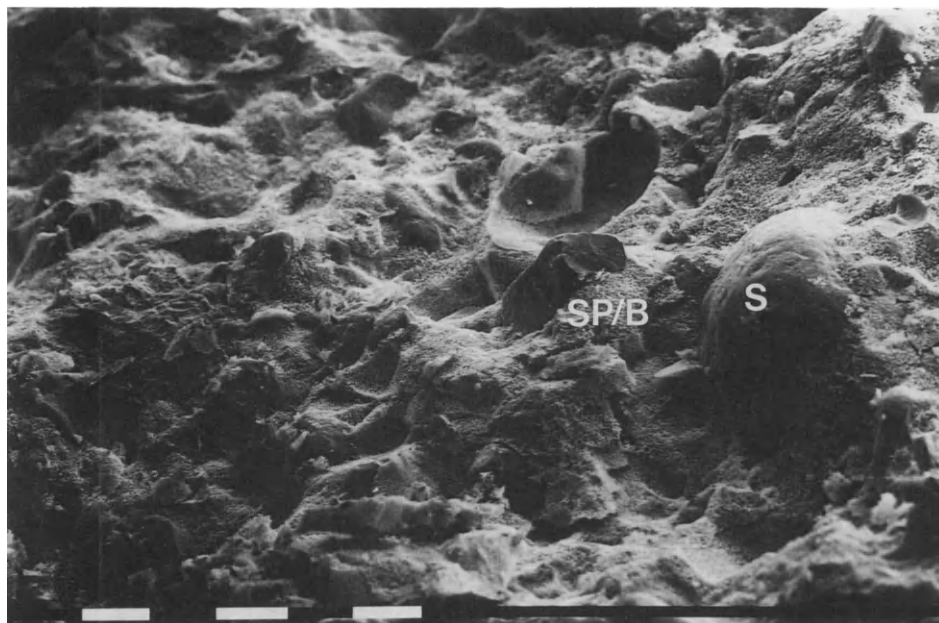


Fig. 5. Loamy sand amended with 5% bentonite, 32% moisture content. S=sand grain; SP=soil plasma; B=bentonite; white bar=0.1 mm.

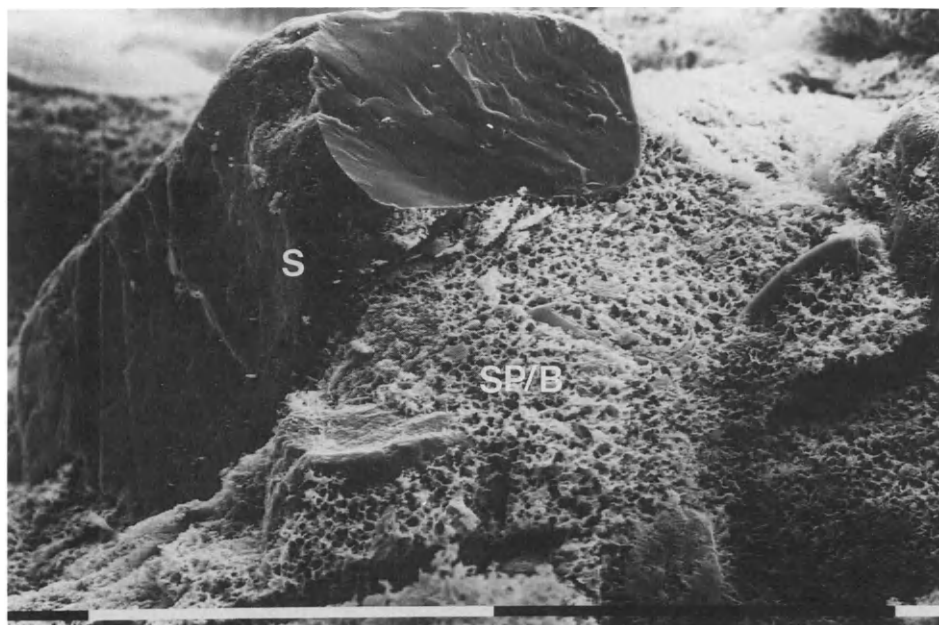


Fig. 6. Loamy sand amended with 5% bentonite, 32% moisture content. S=sand grain; SP=soil plasma; B=bentonite; white bar=0.1 mm.

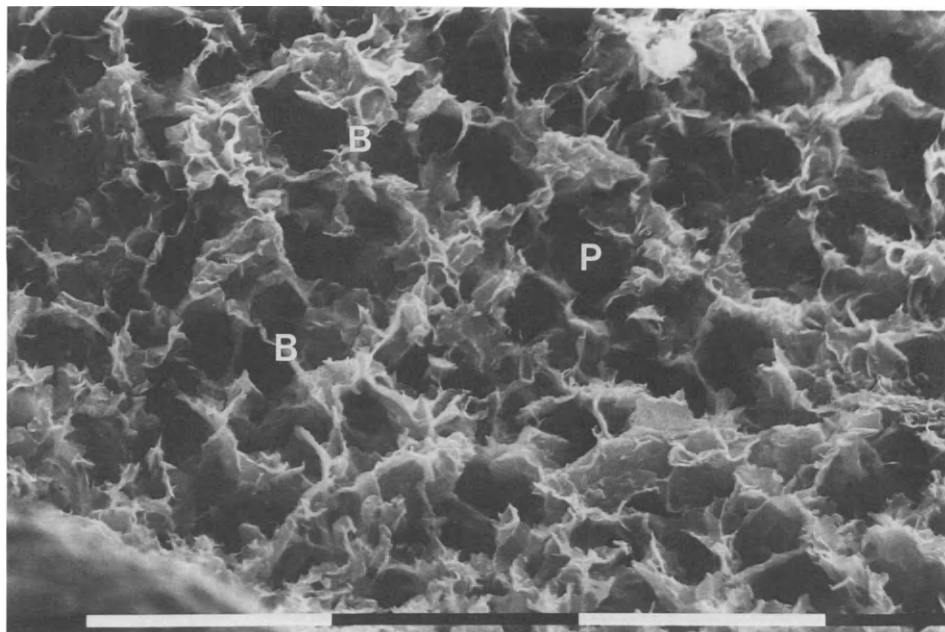


Fig. 7. Soil plasma in loamy sand amended with 5% bentonite, 32% moisture content. P=pore; B=bentonite; white bar = 10 μ m.

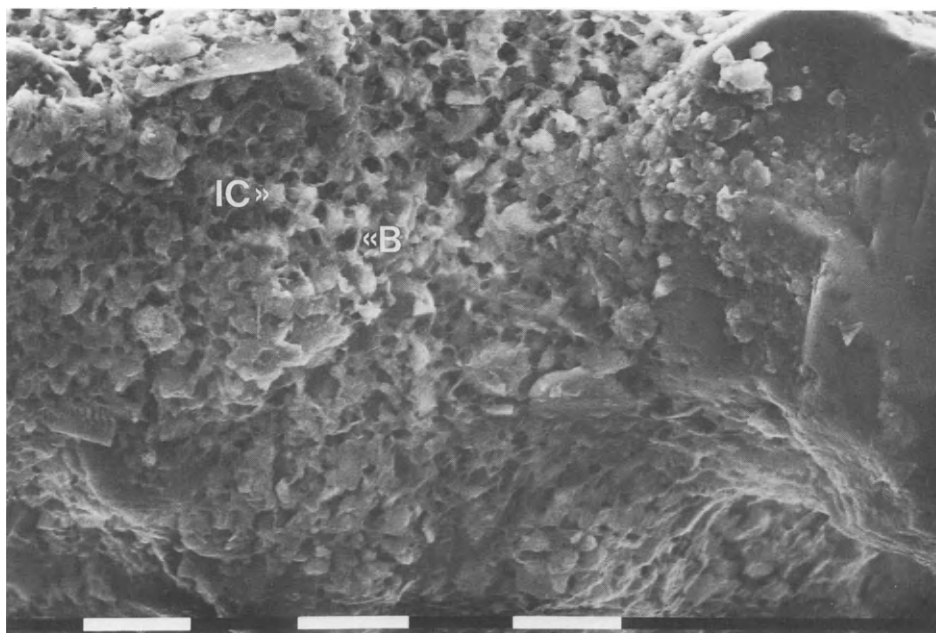


Fig. 8. Soil plasma in loamy sand amended with 1% bentonite. B=bentonite; IC=indigenous clay; white bar = 10 μ m.

(not shown). However, now the pores in the bentonite plasma were much smaller, with maximum pore lengths ranging up to $1\text{ }\mu\text{m}$ only.

After amending the loamy sand with 0.5% or 1% bentonite clay (increasing bacterial survival, but to a less extent than was the case for 5% bentonite, Heijnen et al., 1992) it was more difficult to locate the bentonite in the sample. The clay occurred in thinner layers covering the sand grains, leaving more bare sand grains. Small pores with sizes similar to those observed in the 5% bentonite treatment were detected, but they were less frequent since the bentonite percentage was lower. Bentonite clay and indigenous soil clay often occurred mixed together (Fig. 8). The indigenous clay particles could be recognized by their blocky appearance (as was observed in unamended loamy sand), and were completely surrounded by bentonite. Since bentonite became mixed with indigenous clay when only low concentrations of bentonite were present, it was assumed that this would also happen when higher amounts of bentonite were added to the loamy sand.

DISCUSSION

In previous work (Heijnen et al., 1988, Heijnen and Van Veen, 1991) the survival of *Rhizobium*, introduced into loamy sand, was shown to be improved by the addition of bentonite clay. It was hypothesized that bentonite clay protects introduced rhizobia against predation by protozoa through the creation of many new pores large enough to allow bacteria to enter, but too small to be colonized by predating protozoa. These so-called protective micro-habitats were defined as pores with neck diameters $< 6\text{ }\mu\text{m}$ (Heijnen and Van Veen, 1991). This agrees with Hattori and Hattori (1976), who suggested that pores with diameters between 2 and $6\text{ }\mu\text{m}$ might be favourable micro-habitats for soil bacteria, and with Kilbertus (1980) who suggested that micro-habitats are generally three times as large as the colonizing bacteria [cell measurements of rhizobia range between $0.5\text{--}0.9 \times 1.2\text{--}3.0\text{ }\mu\text{m}$ (Jordan, 1984), but will be smaller according to Postma et al. (1988) after introduction into soil]. In this study we demonstrated that bentonite-amended soil was characterized by the presence of an extremely porous clay matrix. Large numbers of pores were present, with an average maximum length of $\sim 3\text{ }\mu\text{m}$ and an average minimum width of $\sim 2\text{ }\mu\text{m}$ (Table 1). In kaolinite-amended or unamended loamy sand pores with comparable dimensions were also present, but they were less frequent (Table 1) than in bentonite-amended soil. Therefore, the hypothesis that bentonite is responsible for the creation of many small pores ($< 6\text{ }\mu\text{m}$) which could serve as protective micro-habitats for introduced bacteria, was confirmed.

Kaolinite amendments to loamy sand also increased rhizobial survival

compared to unamended loamy sand, but to a less extent than in bentonite treatments (Heijnen and Van Veen, 1991). It is shown that the number of small pores in kaolinite-amended loamy sand is similar to those found in unamended loamy sand (Table 1). However, the pores were slightly smaller (Table 1), offering more protection against predating protozoa which may explain the increased survival levels of bacteria introduced into kaolinite amended as compared to unamended loamy sand (Heijnen and Van Veen, 1991). The inability of kaolinite to create many small pores could be explained by its coarse plate-like structure; large particles cannot be stacked in such a way that many small pores (protective micro-habitats) are created.

Bentonite clay was found to mix well with indigenous soil clay. Also organic matter, surrounded by bentonite, was observed. Especially the latter aspect may be important for bacterial survival, since it means that a food source is situated near the protective micro-habitats.

We could not observe bacteria in the different soil treatments studied, in spite of the fact that at least 10^9 cells were expected to be present in 1 g natural soil. This could be explained by the low colonization percentage (0.1%) of the pore space in soil by bacteria (Adu and Oades, 1978), reducing the chance of detection. Furthermore, if bacteria are covered by clay or organic matter, they are difficult to recognize and might probably not be detected at all. This is in accordance with the fact that on the rare occasions when bacteria were observed in soil, they were usually situated on bare sand grains (C. Chenu, pers. commun., 1991). Therefore, the phenomenon that a large proportion of the soil is not colonized by bacteria, in combination with clay additions and/or organic matter covering the bacteria, could be used to explain the fact that no bacteria were observed in bentonite-, kaolinite- or unamended loamy sand. However, visualization of bacteria in clay with CryoSEM is possible, but only when extremely high bacterial population densities (2×10^{11} cells/g) are present (Schmit and Robert, 1984).

Smectitic clay minerals with polygonally shaped pores and thin wrinkled walls, as shown in Fig. 7, were also found by Tessier (1990). However, the pore sizes of 1–2 μm described by Tessier (1990) were smaller than those which were measured in Fig. 7 (average maximum pore length $\sim 3 \mu\text{m}$). Presumably, freezing artifacts due to the presence of water are not the reason for the occurrence of the large pores (3 μm as compared to 1–2 μm), since the moisture content of the clay studied by Tessier (1990) was higher than in our samples (–3.2 and –10 kPa, respectively). Furthermore, no particular orientation of the pores was observed, which also suggests that freezing artifacts were not a major problem in our studies. However, the pores shown in Fig. 7, and probably also the pores shown by Tessier (1990), might be slightly enlarged due to a decrease of the spacing between layers inside the clay tactoids during freezing from 5–10 to ~ 1.5 nm (Tessier, 1990) (tactoids are particles resulting from the face to face stacking of clay plates; through the interleaving

of plates, particles with a large lateral extension as compared to their thickness are formed). However, this decrease in interlayer spacing, and hence the increase in pore size, is on a much smaller size scale (nm) than that of the pores under observation (μm). Differences in pore sizes comparing our present work and results by Tessier (1990) are probably related to the method of sample preparation. Tessier (1990) equilibrated homogenized clay slurries at given matric potentials. This preparation method allowed for a good dispersion of the clay particles, resulting in small tactoids and, hence, small pores. In the present work, the bentonite was added to the soil as a powder, and after mixing, the sample was brought up to a moisture content corresponding to a matric potential of -10 kPa. The rapid wetting, without an initial dispersion of the clay, will probably cause formation of larger tactoids, and hence the formation of larger pores.

The observed increase in pore lengths from $\sim 1 \mu\text{m}$ to $3 \mu\text{m}$ in the 5% bentonite treatment, when increasing the moisture content from 18 to 32%, could be explained by the swelling of the clay. Wilding and Tessier (1988) showed that, contrary to classical concepts, shrink-swell phenomena can only partly be explained by hydration-dehydration cycles of double layers. Only for Na-saturated low-electrolyte smectitic clay systems the diffuse double-layer model is likely to be functional. In clays with exchangeable Ca and Mg, as in the bentonite clay used here, shrinking and swelling also involves changes in the amount of interparticle pore water, causing size increases or decreases of pores which behave as bellows of an accordion (Wilding and Tessier, 1988). The extent of swelling is determined by the flexibility of the pore walls which in turn depends on the clay mineralogy (smectitic clays provide high flexibility) and the composition of the exchange complex. Flexible pore walls will stretch at high moisture contents, resulting in large pores. In loamy sand amended with 5% bentonite clay, pore sizes indeed increased at higher water contents (compare -80 and -10 kPa).

Swelling and shrinkage of the soil might affect the survival of introduced bacteria in soil due to a fluctuation in pore size. At a matric potential higher than -10 kPa, it is unlikely that pore sizes in the bentonite matrix would exceed $6 \mu\text{m}$, due to the geometry of the pore structure (Tessier 1990). Therefore, at higher moisture contents than corresponding to -10 kPa, protection against protozoa would probably still occur. However, at matric potentials lower than -10 kPa (drier soils), pore sizes would decrease and could reach dimensions smaller than those of bacterial cells. A similar decrease in pore size has been suggested to be detrimental to bacteria in pure clay (Schmit and Robert, 1984). It remains to be studied how the survival of introduced bacteria in soil is affected when pores, colonized with introduced bacteria, are reduced in size due to shrinkage of the soil.

CONCLUSIONS

The method described enabled us to study soil structure on the size scale at which microbial processes are likely to occur in soil. With the use of CryoSEM the hypothesis, that the protection of introduced bacteria in soil by bentonite clay was positively correlated with an increase in the number of small pores, was confirmed. Pores with an average maximum length of $\sim 3 \mu\text{m}$ and an average minimum width of $\sim 2 \mu\text{m}$ were numerous in bentonite-amended loamy sand, offering the introduced bacteria protection from predating protozoa and resulting in high bacterial survival levels.

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Earthworm burrow system development assessed by means of X-ray computed tomography

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ABSTRACT

Joschko, M., Müller, P.C., Kotzke, K., Döhring, W. and Larink, O., 1993. Earthworm burrow system development assessed by means of X-ray computed tomography. In: L. Brussaard and M.J. Kooistra (Editors), *Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships*. Geoderma, 56: 209–221.

Being non-destructive, X-ray computed tomography allows to study the development of earthworm burrow systems with time. Column experiments were carried out with the endogeic earthworm species *Octolasion cyaneum*. The columns (30 cm high, 12 cm wide), filled with loess soil, were scanned after 14, 21 and 92 days. The subsequent three-dimensional analysis of the burrow system revealed the pattern of burrowing and the degree of burrow continuity.

INTRODUCTION

In order to evaluate the soil physical function of earthworm burrow systems, information not only about the spatial arrangement of burrows is essential but also about their potential variability with time.

The development of earthworm burrow systems was studied in the past by using planar glass cuvettes (e.g. Evans, 1947; Bolton and Phillipson, 1976; Martin, 1982; Schrader, 1993). With this technique the three-dimensional soil body is reduced to two dimensions, rendering the burrows visible. Techniques for assessment of the three-dimensional structure of earthworm burrow systems are usually destructive and thus do not permit time sequence

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studies (e.g. Kretzschmar, 1972; Wendt and Larink, 1990; Ligthart, 1993; McKenzie and Dexter, 1993).

Non-destructive techniques for soil analysis include X-rays (Röntgen 1895). They are successfully applied for example to the assessment of soil pore structures (Rogaar and Thiadens, 1975). The potential of X-ray analysis was considerably improved by combination with computed tomography (Hounsfield, 1972, 1980). Since 1982 X-ray computed tomography is successfully applied to soil (e.g. Petrovic et al., 1982; Tollner, 1991). In combination with special data processing techniques soil structure elements assessed by means of X-ray computed tomography may be visualized three-dimensionally, as it was done by Stuyt (1992) in respect to soil macropores around subsurface drains.

Earthworm affected soil was studied with conventional X-ray techniques for example by Rogaar and Boswinkel (1978). X-ray computed tomography proved to be a useful tool for detection of earthworm burrows in the field

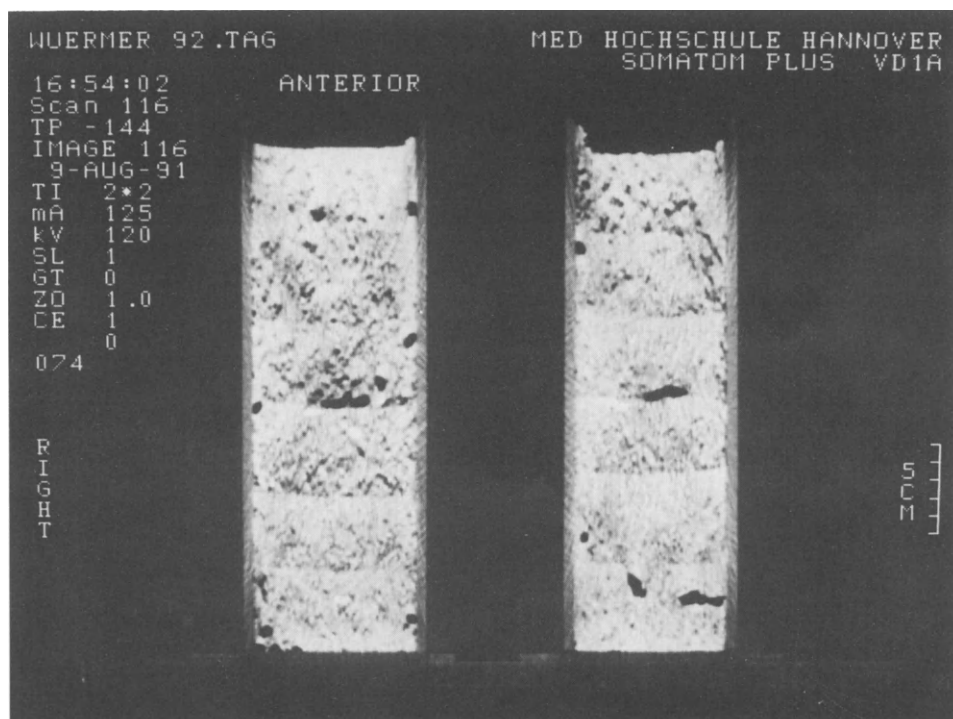


Fig. 1. Scan slice (1 mm thick) of two soil columns with one *Octolasion cyaneum* each; burrows appear black; captions contain informations about technical parameters of the scanning procedure typical for medical computed tomographic scanning.

(Warner et al., 1989) as well as for visualization of three-dimensional earthworm burrow systems in column experiments (Joschko et al., 1991).

Being non-destructive, X-ray computed tomography allows the repetitive scanning of soil samples. Thus the alterations of burrow morphology with time may be studied directly in three dimensions. Tollner et al. have already stressed this potential of the method in 1987. The objective of this study was to test the method of X-ray computed tomography for the assessment of endogeic earthworm burrow development in soil columns.

MATERIALS AND METHODS

Soil columns

Gleyic Chernozem from loess (clay content 26%) was roughly sieved (15 mm) and wetted to a gravimetric water content of 23.2% corresponding to

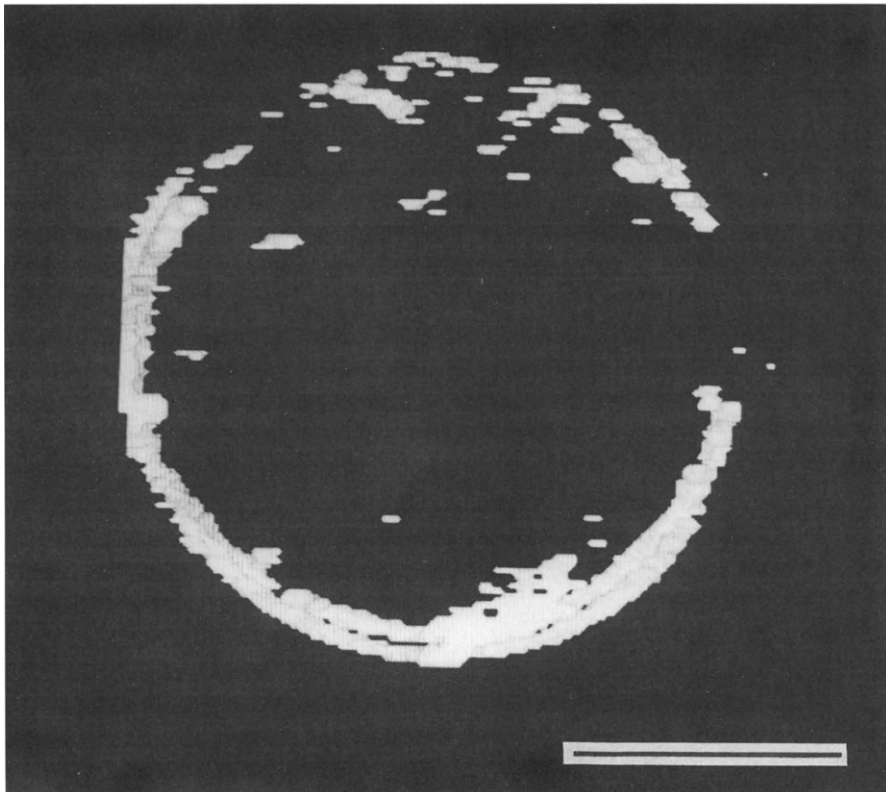


Fig. 2. Burrows of *O. cyaneum* in column 1 at day 21; view from above (bar: 5 cm).

approximately 82% of the maximal water holding capacity. Subsequently the soil was artificially compacted in two plastic cylinders (30 cm high, 12.4 cm wide) in 6 layers each to an average bulk density of 1.44 Mg m^{-3} (column 1) and 1.49 Mg m^{-3} (column 2). Each column was inoculated at the soil surface with one specimen of *Octolasion cyaneum* (Savigny 1826) with a biomass of 1.77 g (column 1) and 1.45 g (column 2). The cylinders were covered with pierced plastic caps to prevent evaporation and stored in a constant-temperature room in the dark at about 12°C .

Computed tomography scanning

The columns were scanned with a Siemens Somatom Plus, located at the University hospital Medizinische Hochschule Hannover (MHH) at days 14, 21 and 92. They were cut in a vertical plane at 120 kV and 500 mAs. Slice thickness was 1 mm; the size of a picture element (pixel) was $1 \text{ mm} \times 1 \text{ mm}$.



Fig. 3. Burrows of *O. cyaneum* in column 1 at day 92; view from above (same scale as Fig. 2).

in a 512-pixel matrix, corresponding to a volume element (voxel) of $1\text{ mm} \times 1\text{ mm} \times 1\text{ mm}$. The data were stored on magnetic tape (Siemens TK 50) and subsequently transferred to a graphic computer located at the Institute of Medical Informatics, University of Hildesheim. Burrow dimensions were calculated from the raw data. The burrows were visualized using a special data conversion program.

Three-dimensional reconstruction of burrows

The following data conversions were carried out: (a) binarization of data matrix, (b) deletion of single and paired macropore pixels, (c) smoothing,



Fig. 4. Burrows of *O. cyaneum* in column 1 at day 21; side-view (bar: 5 cm).

(d) dilation, (e) visualization with AVS program. The procedure is described in detail elsewhere (Joschko et al., 1991).

Quantitative assessment of burrow pattern and total burrow volume

The number of visualized pixels per 1 cm slice of soil column was summed up and plotted versus soil depth. The total number of visualized pixels served as estimation of the total burrow volume per column.



Fig. 5. Burrows of *O. cyaneum* in column 1 at day 92; side-view (same scale as Fig. 4).

RESULTS AND DISCUSSION

In the two-dimensional scan slices the burrows were clearly visible as dark features inside the light soil matrix (Fig. 1). After the three-dimensional reconstruction the burrows appeared white (Figs. 2–5 and 7–9). Besides the earthworm burrows some other soil pores were visualized as they could not be separated from the burrows by the reconstruction procedure. The spatial arrangement of burrows could be elucidated by comparing the three-dimensional reconstructions in different projections. In the view from above the burrowing had followed the pattern (a) burrowing along the wall, (b) invasion into the column center (Figs. 2 and 3). This pattern was similar in both columns. The preference of earthworm burrowing along the walls in column experiments has frequently been described (e.g. Kretzschmar, 1990) but could not be visualized yet in endogeic earthworm species.

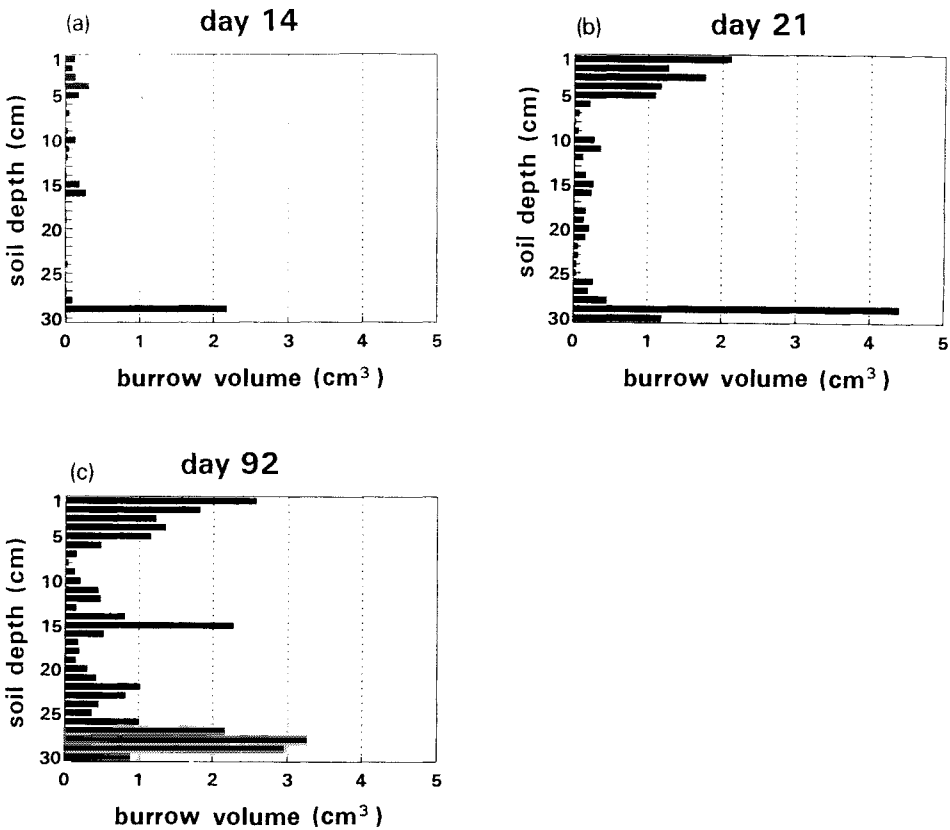


Fig. 6. Scheme of burrow development of *O. cyaneum* in column 1: (a) 14 days, (b) 21 days, (c) 92 days.

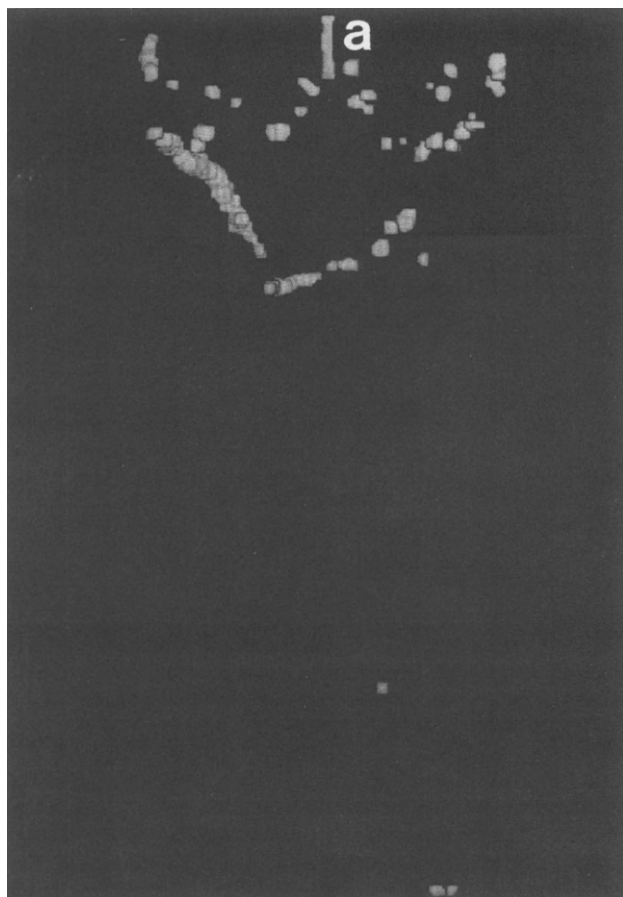


Fig. 7. Burrows of *O. cyaneum* in column 2 at day 14, side-view; *a* = artificial hole which had been applied to the soil surface to facilitate the earthworm burrowing into the soil (same scale as Fig. 4).

In the side-view the pattern of burrow development appeared to be different in both columns (Figs. 4–10). In column 1 the worm had first burrowed to the bottom of the column. From there the burrows extended into the medium part of the column (Figs. 4–6). This pattern of burrow development corresponds to observations of Bolton and Phillipson (1976) for the endogeic species *Allolobophora rosea*: in glass cuvettes the worms burrowed at first to the bottom of the cage, then followed the bottom edge and ascended up to the surface. Subsequently short side burrows were created which ramified into the soil (Bolton and Phillipson, 1976). A similar pattern of burrowing was described by Evans (1947) for *Allolobophora caliginosa*.

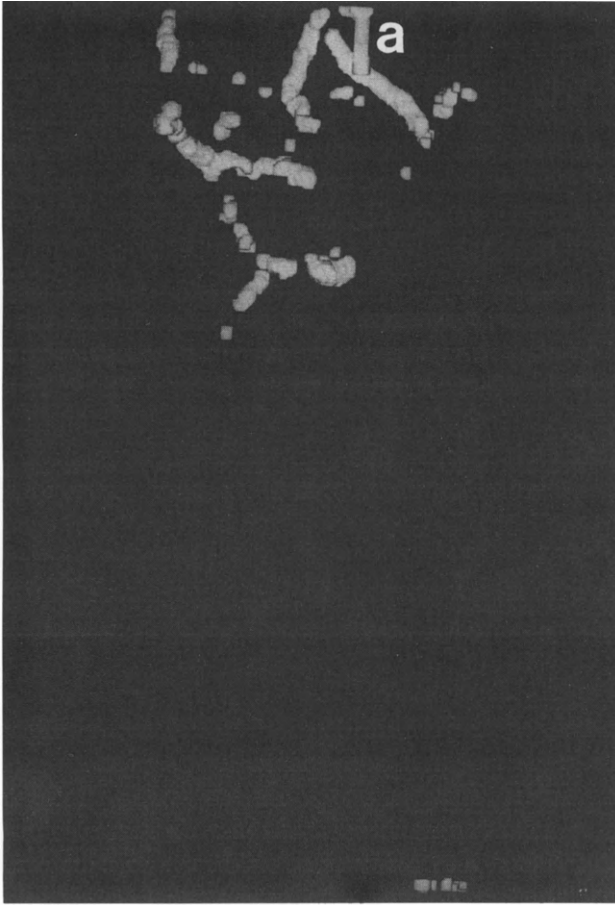


Fig. 8. Burrows of *O. cyaneum* in column 2 at day 21; side-view; a=artificial hole, same as in Fig. 7 (same scale as Fig. 4).

In column 2, the U-shaped burrow system was at first restricted to the upper half of the column (Fig. 7). At day 21 it showed some ramifications into the inner soil part (Fig. 8). Between day 21 and 92 the burrow system shifted to the lower half of the column (Figs. 9 and 10). During this procedure the old burrows must have largely been filled up with casts, since only small parts could be detected in the upper half of the column after 92 days. For deposition of egested soil not only old burrows were used but also an artificial hole which had been applied to the soil surface (Figs. 7–9).

Possible reasons for this burrow pattern are bulk density differences within the column. The compaction procedure led to six compacted layers which can be identified in the X-ray computed tomography scan slices (Fig. 1). The



Fig. 9. Burrows of *O. cyaneum* in column 2 at day 92; side-view (same scale as Fig. 4).

vertically oriented dense layers are more marked in column 2, which had a slightly higher average bulk density (Fig. 1). An explanation for the observed burrow pattern in this column is that only after day 21 the worm succeeded in penetrating a dense layer in the upper half of this soil column.

A complete shift of the burrow system from one part to another of an experimental set up was observed by Bolton and Phillipson (1976) in *A. rosea*. The X-ray analysis thus confirmed observations in the three-dimensional space which have previously been found in experiments with glass cuvettes. In contrast to the planar glass cuvette technique, however, X-ray computed tomography allows to analyze the burrow morphology and its variation with time in the vertical *and* in the horizontal plane. Comparing the burrow development in both columns, a basic pattern could be identified despite the apparent differences between the two columns: the worms first burrowed to the outer limits of their immediately accessible environment, then burrowed into the center.

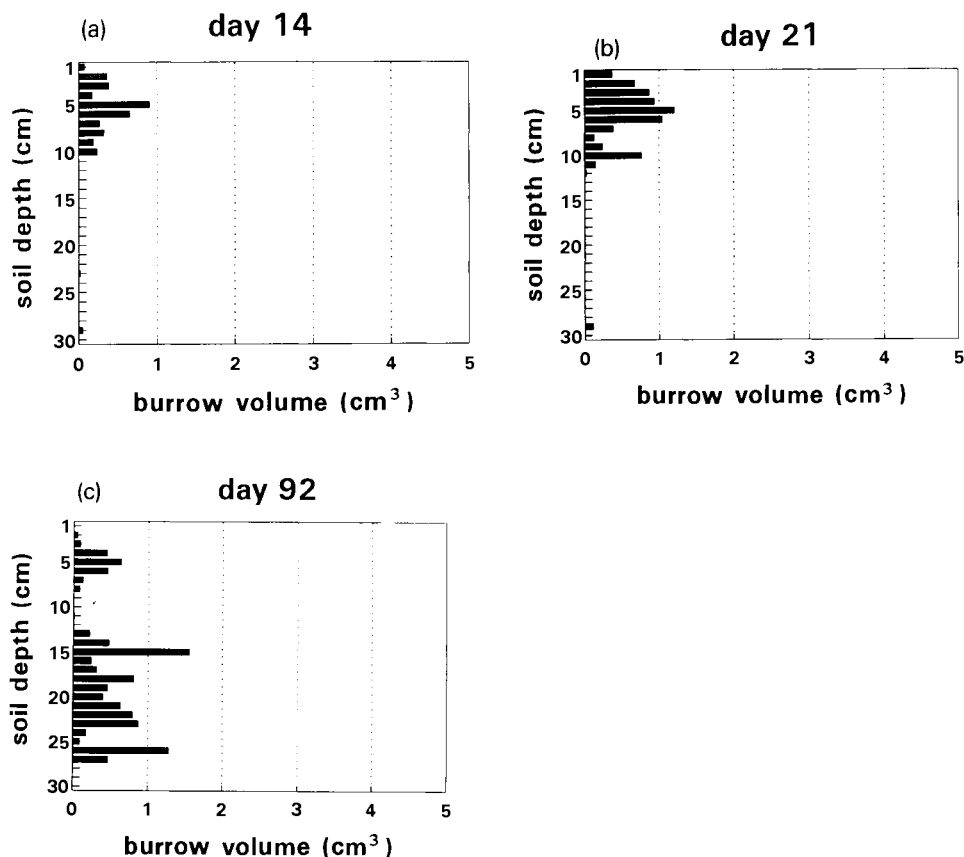


Fig. 10. Scheme of burrow development of *O. cyaneum* in column 2: (a) 14 days, (b) 21 days, (c) 92 days.

The quantification of the open burrow volume consists in summing up the burrow pixels per column. The estimated total burrow volume was 27.8 cm^3 in column 1 and 10.6 cm^3 in the slightly more compacted column 2 after 92 days. The rate of burrow formation appeared to decline over time, a phenomenon which has frequently been described in earthworm experiments (e.g. Bolton and Phillipson, 1976).

With respect to the burrow volume estimation two sources of errors may be pointed out. The total number of pixels includes not only burrows but also other soil pores which could not be separated by the employed data processing techniques. This may lead to an overestimation of burrow volume. On the other hand it was not possible to distinguish between unaffected soil and ingested and egested soil, i.e. casts by the scanning procedure. X-ray computed tomography is thus suited to estimate the actual open burrow volume but not necessarily the total burrow or cast formation rate of the worms. In this re-

spect the planar glass cuvette technique has the advantage that by direct observation it is well possible to distinguish between original soil and casts (e.g. Bolton and Phillipson, 1976; Schrader, 1993).

The continuity of the burrows was low in both columns at all times. Open burrow parts were separated by soil plugs, i.e. casts, which could not be distinguished from the original soil and which appeared as gaps in the three-dimensional pictures (Figs. 4, 5 and 7–9). It means that the burrows in both columns were at least partially produced by ingesting soil and not only by pushing soil aside. The relative contribution of each mechanism of burrow formation remains unclear.

CONCLUSIONS

Our experiment has shown that X-ray computed tomography is a useful tool for studying qualitatively and quantitatively the distribution of earthworm burrows not only in space but also in time.

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Roughness of soil pore surface and its effect on available habitat space of microarthropods

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ABSTRACT

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Like the majority of natural objects, soil pores can be described by means of fractal geometry. Since the area of a fractal surface depends on the scale of measurement, the amount of available pore area for different size classes of non-burrowing soil animals should depend on the fractal dimension of the soil pore surface following the relationship $A(\delta) \approx \delta^{2-D_s}$, where A is the area measured on scale δ , and D_s is the fractal dimension of the surface. We estimated D_s by photographing and digitizing thin sections of various Austrian soils. Images were analyzed using the area-perimeter method following the relationship $P \sim A^{D_p/2}$, where P is the perimeter of an intersected soil pore measured at any convenient scale, A is its sectional area, and D_p is the fractal dimension of the boundary line. $D_p/2$ can be recovered by a linear regression in a plot of $\ln P$ against $\ln A$. Under the assumption of isotropy adding 1 to D_p yields the estimate of the fractal dimension of soil pore surface. Our estimates of $D_s \approx 2.3$ suggest that a decrease in an order-of-magnitude of body length will increase available habitat-space approximately 4 times. We discuss the usefulness of the measurement of pore-wall roughness for predicting a size-class abundance/body size relationship in soil micro-arthropod communities.

INTRODUCTION

In the past two decades much emphasis in ecological research has been laid on the analysis of the relationships between the number, abundance and size of species in natural communities. Numerous papers have been published on relations between two of these community variables (e.g. May, 1975; Griffiths, 1986; Brown and Maurer, 1986; Lawton, 1989). Only until recently has the shape of a three-dimensional surface been investigated, i.e., one defined by the three variables as given above (Harvey and Lawton, 1986; Morse et

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al., 1988; Blackburn et al., 1990). Little information exists on the species number/abundance distribution in soil animal communities (Usher, 1985; Vegter et al., 1988; Kampichler, 1992), and even less on the other relationships (e.g. Haarløv, 1960). This paper concentrates on the importance of certain physical properties of the soil environment for predicting a size-class abundance/body size distribution in soil microarthropods.

Several studies have dealt with fractal concepts in order to describe or to explain a variety of pedological features (e.g. Ahl and Niemeyer, 1989; Tyler and Wheatcraft, 1989; Toledo et al., 1990; Bartoli et al., 1991; Young and Crawford, 1991). Since we aim at an analysis of available habitat space for a group of non-burrowing animals which are restricted to the surfaces of existing air-filled crevices in soil, we confine our investigation to the estimation of the fractal dimension of the pore walls*.

In general, the mass M of a fractal object (length, surface, volume) depends on the scale of measurement δ , following the relationship:

$$M(\delta) \approx \delta^{d-D} \quad (1)$$

where d denotes the Euclidian dimension, and D is the fractal dimension. Area A of a fractal surface is measured as follows:

$$A(\delta) \approx \delta^{2-D} \quad (2)$$

Consequently, the amount of available pore area for individuals of non-burrowing species of different sizes depends on the fractal dimension of the pore walls (cf. Sugihara and May, 1990). As A increases with decreasing δ , usable area increases for smaller individuals. Hence small-sized individuals should be more abundant than large-sized individuals. Apart from other influencing factors we may expect that the rougher the pore walls in a given soil are — i.e., the higher their value of D — the steeper will be the size/abundance distribution of micro-arthropods. Morse et al. (1985) have shown that real data on the distribution of arthropod body lengths are broadly consistent with predictions based on the fractal dimension of the vegetation surface. From this point of view we must consider the estimation of the roughness of the soil pore surface necessary for developing a model on the relationships between number, body size and abundance of species of soil microarthropods.

METHOD

Image analysis and fractal geometry

When an isotropic three-dimensional soil sample is intersected by a plane,

*For an introduction to fractal geometry and its application in ecology see Fortin (1987) and Sugihara and May (1990).

then the intersected soil pores can be identified as two-dimensional patches (see qualifying comments below). A simple relationship exists between the fractal dimension of a patch perimeter D_p and the corresponding pore surface dimension D_s (cf. Mandelbrot, 1987, pp. 227–228):

$$D_p = D_s - 1 \quad (3)$$

Hence the analysis of D_s can be performed on the basis of image analysis of thin sections of soil (Protz et al., 1987; Ringrose-Voase, 1987; Moran et al., 1989; Bartoli et al., 1991).

To estimate the boundary dimension of patches, the relation between area and perimeter (the so-called area–perimeter or A – P relation) served as a convenient method (Lovejoy, 1982; Krummel et al., 1987; Mandelbrot, 1987).

TABLE 1

Locations, pedological features and depth of soil samples used for the thin sections^a and results of the regression $\ln P = D_p/2 \times \ln A + \ln K$

Sections	$D_p/2$	S.E.	R^2	D_s
Hermannsdorf (Upper Austria) Arable land Stagnogley ($\approx$ Stagno-Gleyic Luvisol) Loamy silt Soil horizon Ag, 0–2 mm	0.70	0.02	0.96	2.39
Hermannsdorf (Upper Austria) Arable land Stagnogley ($\approx$ Stagno-Gleyic Luvisol) Loamy silt Soil horizon Ag, 2–9 mm	0.63	0.03	0.93	2.26
Kaltenleutgeben (Lower Austria) Deciduous forest Terra fusca ($\approx$ Chromic Cambisol) Soil horizon Ah, 0–5 cm	0.67	0.01	0.96	2.33
Kaltenleutgeben (Lower Austria) Deciduous forest Terra fusca ($\approx$ Chromic Cambisol) Soil horizon Bv, 12–16 cm	0.66	0.01	0.96	2.32
Trautenfels (Styria) Arable land Pararendzina ($\approx$ Calcaric Regosol) Loamy sand Soil horizon Ah, 10–20 cm	0.65	0.01	0.93	2.30

^aAustrian soil taxonomy after Fink (1969) with corresponding units of the FAO system according to ISSS (1986) and Scheffer and Schachtschabel (1989), symbols of the soil horizons after Fink (1969).

The A - P relation is a measure of the contortion of the perimeter. A fixed length of smooth or rounded perimeter can enclose a larger area than a complicated one. The degree of roughness can be qualified by the dimension D_p of the perimeter, which is given by the solution of:

$$P \sim A^{D_p/2} \quad (4)$$

For smooth shapes $D_p = 1$, and $P \approx A^{1/2}$, whereas for highly contorted shapes, where the perimeter attends to fold back on itself thus filling the plane, and D_p approaches 2.

After calculating the perimeter P and the area A of each patch at some fixed δ at a pixel basis, the values of P against A are plotted on a double logarithmic scale. The slope of the regression line resulting from eq. (4) in the logarithmic form equals $D_p/2$.

Production and processing of images

The thin sections studied were derived from various localities in Austria (Table 1), and produced by the method described by Altemüller (1962). Photographic images of three to five sectors of each section were made with an ILFORD PAN-F film, developed with maximum contrast and magnified (Fig.

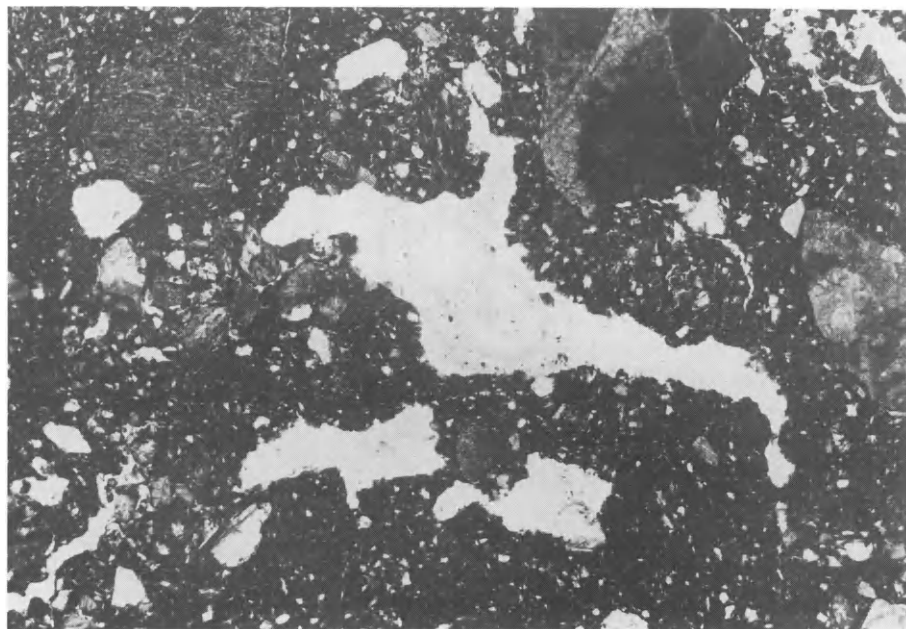


Fig. 1. Photograph of a sector of a soil thin section from arable land (Trautenfels, cf. Table 1). Size of the sector: 4 mm $\times$ 6.4 mm.

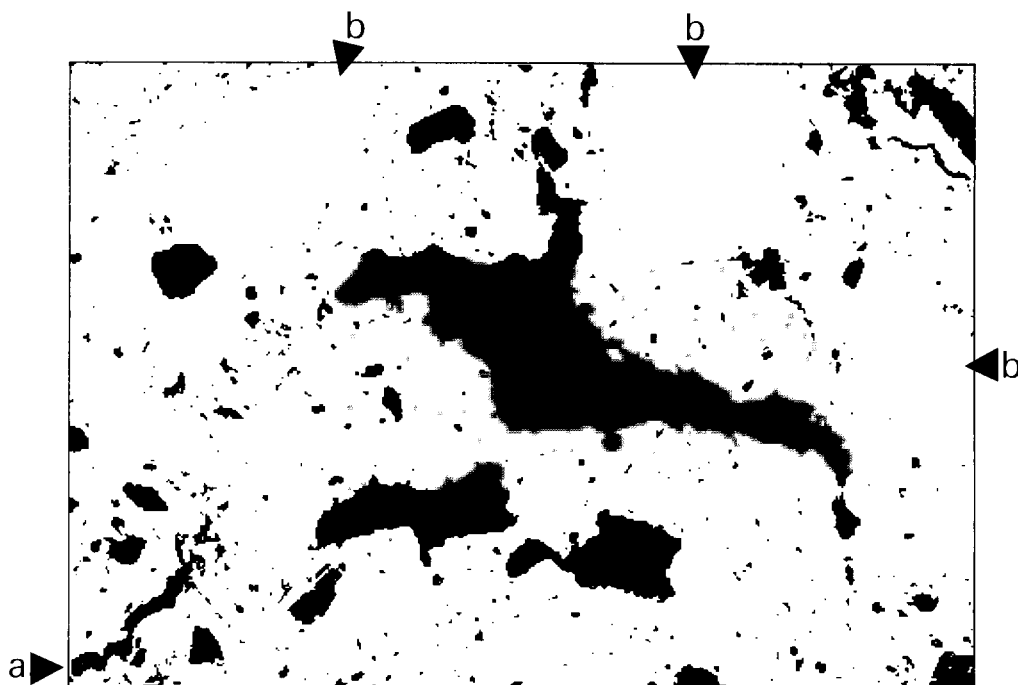


Fig. 2. Digitized image of Fig. 1 (see text for explanation).

1). Then the images were scanned with a MICROTEK MSF-400G scanner into a MACINTOSH *cxII* computer with a band width of 1 bit (in order to obtain only either black or white areas) at a resolution of 72 dpi (dots per inch). The smallest discernible unit (pixel) of the digitized images corresponded to 6 or 12 μm , depending on the sector size. The pores were now represented as contiguous areas of white color, whereas the soil was black. Using a painting program the images were inverted, so that the pores were now black and the soil white (Fig. 2).

The following corrections were done on the digitized images:

(1) All patches of pores that crossed any of the edges of the picture were excluded from the analysis, as then a part of the perimeter of those pores is a straight line and distorts the overall calculation. Exceptions were made when the length of the section line was negligibly short in comparison to the total perimeter of the pore. In that case the straight section line was replaced by a piece of original pore perimeter of the same length (see arrow *a* in Fig. 2).

(2) Large pores with narrow interconnections of one or two pixels width were separated.

(3) After scanning mineral grains in the soil sections appeared as spotted areas. They were removed completely (see arrows *b* in Fig. 2).

(4) A change in dimension with size scale may indicate a shift in generating

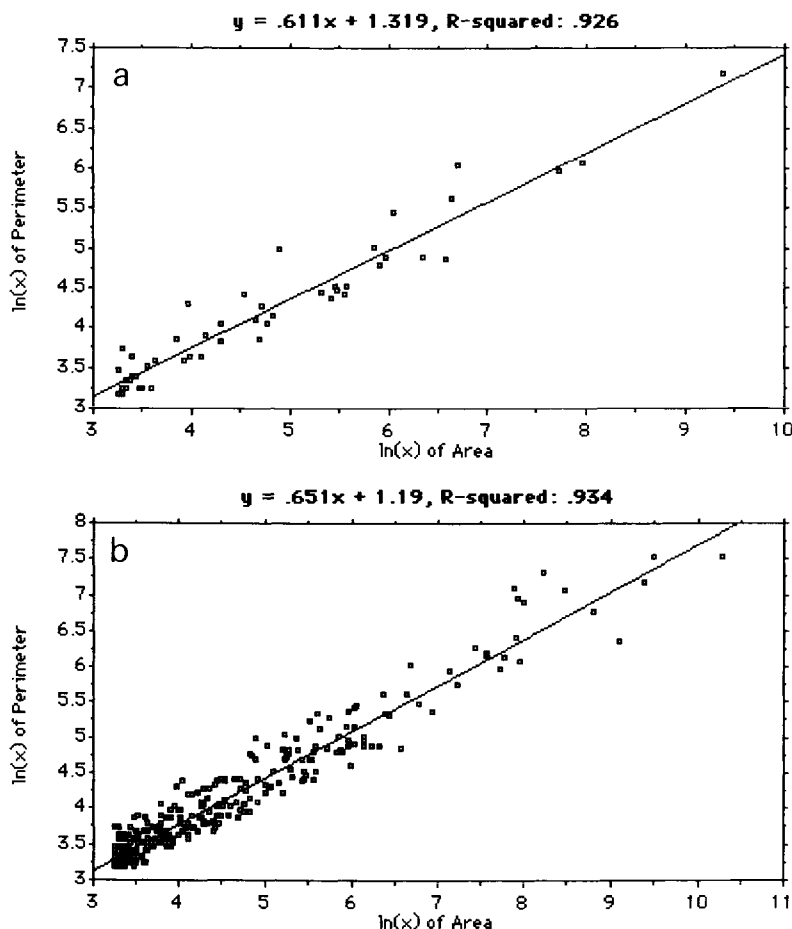


Fig. 3. Regression of $\ln P = D_p/2 \times \ln A + \ln K$. (a) Regression based on the digitized image of Fig. 2. (b) Regression based on pooled data from Fig. 2 and three additional photographs from the same thin section. Both axes in pixel units; length of 1 pixel = 12 μm .

process and is indicated by a kink in the regression line (Sugihara and May, 1990). In contrast to Bartoli et al. (1991), this study restricts itself to soil pores that actually are habitable to micro-arthropods, in order not to mix together pore wall dimensions originating from different soil forming processes. Therefore, a lower threshold was established at a patch area of approximately 0.003 mm^2 . Thus, only pores having an area of at least 0.003 mm^2 and larger were analyzed.

The so retouched images were then processed by a specially designed computer program that measures P and A of every distinguishable patch. Using a statistics program the linear regression was carried out (Fig. 3).

The application of the P - A relation method is restricted to specific soil con-

ditions. For example in a two-dimensional section the identification of pores as discrete patches should be possible. Sections that show an interwoven network of soil matter and crevices cannot be analyzed by the computer program used here.

RESULTS

Table 1 presents the results of the regression of $\ln P$ on $\ln A$. As stated in eq. (3), adding 1 to D_p yields the fractal dimension of the pore surface D_s . Although originating from different soils, D_s varies only slightly around 2.32.

We inserted this result into eq. (2) to estimate the available area for microarthropods of different size. After a reduction in linear body size by the factor 0.1 the reduction in individual "cover" (= area of the vertical projection of a specimen upon the substratum, introduced by Haarløv, 1960) can be estimated by squaring (cf. Morse et al., 1985). Thus, we assume a $(0.1 \times 0.1)^{-0.32} \approx 4$ -fold increase in available habitat space. Accordingly an order-of-magnitude decrease in body length should result in a 4-fold increase in density of individuals on a given pore area.

DISCUSSION AND CONCLUSIONS

Usefulness of the method

As stated above the A - P relation can only be used when specific presuppositions are satisfied. The strict criteria on the selection of soil sections might by all means be responsible for the narrow range of D_s ascertained in this study. In particular the need for fractal analysis of organic layers — where the most diverse soil microarthropod communities can be found — will set a limit to the applicability of this method. Further analysis of the boundary dimensions of soil pores should be based on a grid method or the "flexible yard stick" method (Vlcek and Cheung, 1986; Tatsumi et al., 1989; Sugihara and May, 1990).

Implications for size class distribution

The fractal dimension as a measure for the roughness of soil pore walls constitutes the first step in the development of a theoretical account of the abundance/body size distribution. It predicts available habitat space not for species but for individuals of a certain size and may therefore help to predict the size-class abundance/body size distribution (cf. Morse et al., 1988, for the differences and connections between these types of relationships). Inserting D_s into eq. (2), calculating available habitat space and drawing inferences from the result about abundance of microarthropods of different size sup-

poses that all soil crevices are accessible to all soil micro-arthropods, from the smallest to the largest. This obviously cannot be the case. Techniques of image analysis should bring about a correction by investigation of the share of pore diameter classes on total pore area, which could yield a factor of pore accessibility for any size class of microarthropods.

Further investigations will have to concentrate on the variability of fractal dimension of habitable pore surface in time and space. Particularly in organic layers we expect seasonal changes and high spatial variability due to comminution and decomposition of plant debris. Collembolles with their high reproductive rate and their ability of synchronized reproduction (Joosse, 1983) may adjust to changing habitat features rather rapidly. Compared with this oribatid mites have a lower reproductive rate, need several months for development and show only moderate changes in population size (Butcher et al., 1971; Petersen, 1982). Data on velocity and extent of changes of D_s may enable us to answer if microarthropod communities or at least certain taxa actually equilibrate with the geometric features of their habitats.

It also must be remembered that a specific relation exists between abundance of animals and their metabolic rates that influences the size-class abundance:body size distribution. Metabolic rates of meso- and macrofauna in soil and litter scale to the power of 0.813 with respect to body weight (Ryszkowski, 1975). Hence, if animal abundance is reciprocal to metabolic rate (Morse et al., 1985; Damuth 1987), a decrease in body size at an order-of-magnitude will result in an increase of density by a factor of $0.1^{3 \times (-0.813)} \approx 275$.

The multiple effects of soil pore roughness, accessibility of pores per size class and body-size:metabolic-rate relationship yield a simple model for a theoretical consideration of the size-class abundance:body size distribution, which can be verified in a subsequent check of the data. The next step will be to produce a comprehensive account on body size, abundance and number of species in microarthropod communities from the organic layers of a spruce forest and from arable land.

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Size and orientation of burrows made by the earthworms *Aporrectodea rosea* and *A. caliginosa*

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ABSTRACT

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The morphology of surface-opening burrows made by earthworms in a South Australian duplex Red-brown Earth was investigated. Coordinate points along burrows were measured during careful excavation. Burrow length, orientation and the frequency of branching were measured. Mean burrow length was 398 mm (s.d. = 189) with typically 2 to 3 branching points. Polar coordinate representation of the burrows showed them to be nearer to vertical at greater depths. Maximum depth of excavated burrows was approximately 250 mm, placing them almost entirely in the soil A horizon.

INTRODUCTION

Earthworms move through soil, creating burrows. Lee (1985) has described three principal forms of burrows. These are usually vertical burrows, sometimes with branching near the surface as described by Lamparski et al. (1987); burrows of geophagous species that forage for food in the subsurface soil horizons but which are predominantly horizontal with some surface openings; and the more or less vertical burrows made by surface-living earthworms as a retreat during dry or cold seasons. The intensity of burrowing activity by geophagous species is related to food supply (Martin, 1982) as well as temperature and water availability.

Burrows, as with biopores in general are cylindrical channels and have been described as important for water and air movement through soil (Ehlers, 1975) and possibly provide paths for plant root growth (Dexter, 1986; Jakob-

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sen and Dexter, 1988). The significance of the size, orientation and longevity of single burrows formed by geophagous species has not been extensively examined although Kretzschmar (1982, 1988) has studied seasonal variations and used simulations to describe burrow systems. Kretzschmar (1978, 1982) found that different ecological groups of earthworms contribute to the different burrow forms and that the quantity of burrows varied through the year and was well correlated with the soil water content and temperature. He found most burrows between 20 and 40 cm deep in the soil. To describe their orientation he used the terms sub-horizontal and sub-vertical.

Data on the numbers of burrows per unit area and the diameters of these burrows have been presented by Barley (1959), Ehlers (1975) and Omoti and Wild (1979). Rogaar and Boswinkel (1978) examined burrows in a polder with a mixed earthworm population and noted two types of burrows; predominantly vertical burrows up to 8 mm wide continuing to depths approaching 1 m, and twisting burrows of 0–8.5 mm diameter down to a maximum of 270 mm. Vertical burrows were attributed to *Lumbricus terrestris*, while the less deep burrows were attributed to several species, including *Aporrectodea caliginosa* and *A. rosea*.

The objective of this study was to examine the suitability of using coordinate points derived from excavation of burrows as a method of quantifying burrow architecture and to use geometrical representation of the data obtained to quantify changes in length and direction of burrows.

MATERIALS AND METHODS

Site and soil

The site chosen for investigation was in a permanent pasture of *Trifolium subterraneum* L. and grasses growing in the Urrbrae fine sandy loam of the Red-brown Earth group (Oades et al., 1981) at the Waite Agricultural Research Institute (34°58'S, 138°38'E).

The A1 horizon has 17% clay ($< 2 \mu\text{m}$), contains no calcium carbonate and extends to a depth of approximately 150 mm. It lies over an A2 horizon extending a further 150 mm (Oades et al., 1981) below which is a clay (65% $< 2 \mu\text{m}$) B horizon. There had been no tillage at the site for many years but super-phosphate fertilizer had been broadcast 4 months before the commencement of this study. The area has a Mediterranean type climate with a warm, dry summer and a cool, wet winter. Most of the mean annual rainfall of 586 mm falls between May and October. The area was not irrigated. Soil temperatures at 150 mm depth range from a mean daily maximum of 30.3°C in January to a mean daily minimum of 9.7°C in July (Waite Agricultural Research Institute, 1987). The excavation of burrows reported here was done between August and mid-October, 1986.

Method for mapping surface-open burrows

An aluminium framework $1.2 \text{ m} \times 1 \text{ m}$ (Fig. 1) was placed flat on the soil surface. In tracks along the sides 1 m apart a trolley could be moved (in the x -direction). A wheel-mounted pointer on the trolley could be moved (in the y -direction) at right angles to the direction of trolley movement. The pointer which could be lowered and raised (in the z -direction) could be fixed in place with a screw. Metal tapes marked at 1 mm intervals were fixed to the framework. Moving the trolley and the pointer on the horizontal plane enable the x and y coordinates to be determined, with the vertically moving pointer allowing the z coordinates to be read from a scale. The entire framework weighed approximately 25 kg and could be dismantled for easy transport.

When in use, the framework was first positioned, and then the soil surface was examined for any biopores opening to the surface. The presence of cast material on the soil surface was used to locate likely starting points. Only burrows open to the surface were mapped. Once a surface opening of a burrow had been found, the above ground parts of the vegetation in the immediate vicinity were removed using scissors. Care was taken not to disturb the soil.

After the coordinates of the surface opening had been recorded, mapping of the burrow proceeded by gently inserting a dissection probe a short dis-

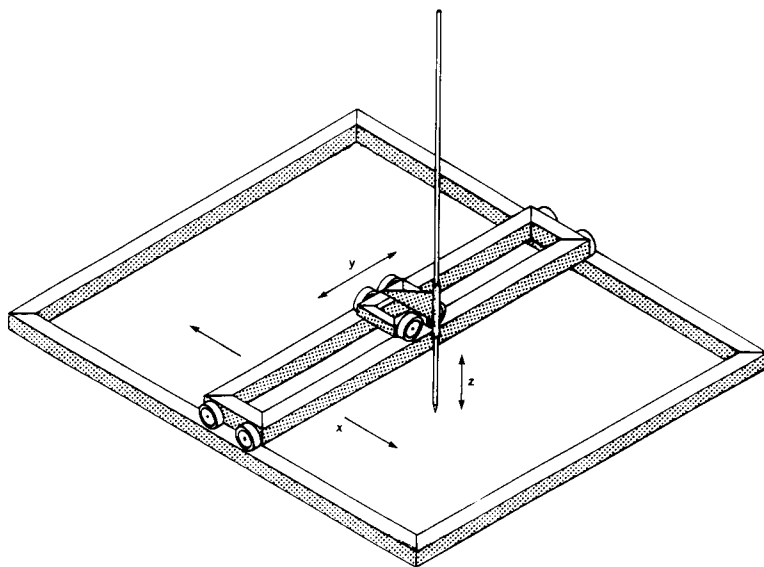


Fig. 1. Aluminium framework used for mapping earthworm burrows showing the trolley for movement in the x -direction, and the wheel mounted pointer for movement in the y - and z -directions. Dimensions are 1.2 m by 1.0 m. Pointer length 1.0 m.

tance into the biopore, or until resistance was detected. The soil was then excavated around the probe using spatulas and fine brushes. The coordinates of the newly exposed point were recorded and the procedure repeated. Coordinate readings were taken in approximately 15 mm segments. Earthworms found in the burrow were collected, anaesthetized and returned to the laboratory for identification. The coordinate data were then transferred to computer disc. As there was some skewing of the trolley as it was moved, the x coordinates were measured at each end of the trolley and the mean value calculated to overcome the parallax. From the x , y , z coordinates the length and orientation of each segment of the burrow were calculated.

Techniques for geometrical description of linear and planar features in soil have been reported by Willoughby (1967). The technique is readily applicable to the description of burrows created by earthworms particularly if (x , y , z) coordinate measures are available of burrows.

RESULTS AND DISCUSSION

Sixteen burrows (open to the surface and containing at least one adult earthworm) were mapped in their entirety. For these burrows the mapping process was not stopped by an inability to follow the burrow (e.g. around plant roots) or because the excavation process destroyed unmapped parts of the burrow. Rovira et al. (1987) observed during sampling of *A. caliginosa* populations that many small worms were associated with plant roots. Similar associations were a major cause of difficulties in mapping burrows here. For each successful burrow excavation there were more than five incomplete excavations. Only one burrow contained more than one adult worm.

The mean length of the burrows was 392 mm (s.d. = 189). All the burrows were produced by either *A. rosea* or *A. caliginosa*, ten by the former and six by the latter. The only other species found at the site was *Microscolex dubius*. As suggested by Lee and Foster (1991) it was not possible to correlate any macroscopic measurements of soil physical properties such as water content with individual earthworms. Similarly no correlation between the fresh weight of the earthworms and burrow length or depth was found.

The number of surface openings per burrow is shown in Fig. 2. The mean number of surface openings per burrow is 3.2 (s.d. = 1.8). The mean number of branches (i.e. points from which more than two paths lead) per burrow was 2.7 (s.d. = 2.0). The majority of branches were in the top 50 mm of soil (Fig. 3). The numbers of surface openings and branches were both positively correlated (at the 5% level) with burrow length. The distribution of burrow length with depth is shown in Fig. 4 with the greatest depth of 195 mm.

The angle and length of burrows occurring in 50 mm layers from the soil surface is shown in Figs. 5, 6 and 7. These were plotted using the technique of Willoughby (1967). The burrow sections are divided into 5° increments and

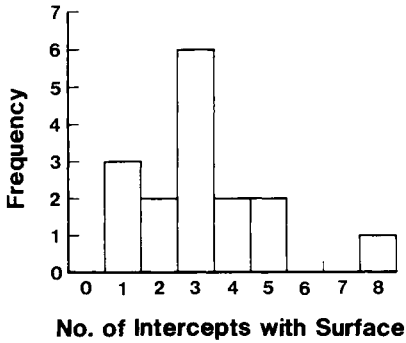


Fig. 2. Histogram of the number of surface openings per burrow.

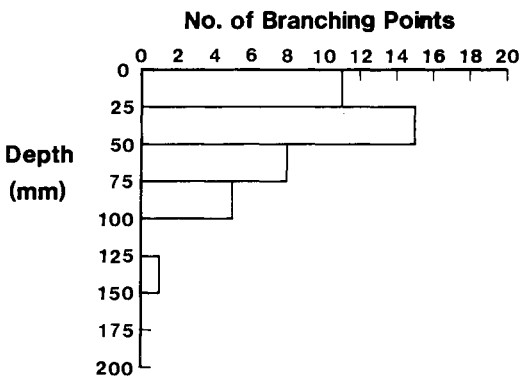


Fig. 3. Number of burrow branching points as a function of depth, in 25 mm increments.

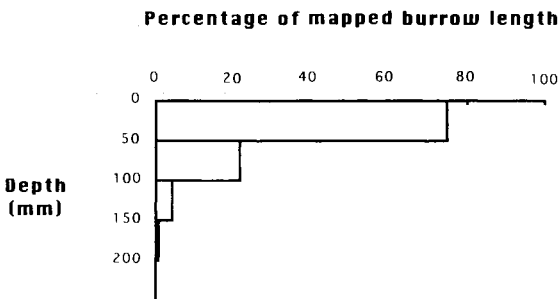


Fig. 4. Percentage of burrow length as a function of depth, in 50 mm increments.

the total length of burrow in each increment plotted. As only 53 mm of burrow was mapped below 150 mm depth these are not graphed, but the mean angle of these burrows was 61.3° measured from the horizontal. Figure 8 shows the relationship between the mean angle, θ , that the burrow makes with the horizontal and the mean depth, z (mm), of that layer. Regression yields:

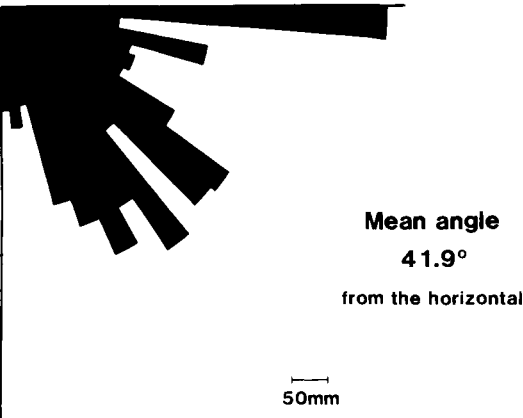


Fig. 5. Polar coordinate representation of burrow length and angle in 5° increments, for 0–50 mm soil depth.

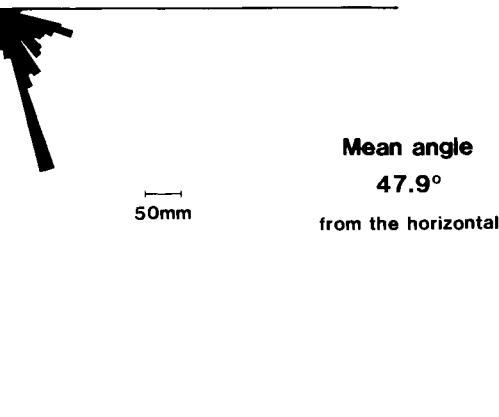


Fig. 6. Polar coordinate representation of burrow length and angle in 5° increments, for 50–100 mm soil depth.

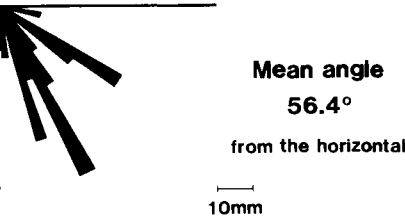


Fig. 7. Polar coordinate representation of burrow length and angle in 5° increments, for 100–150 mm soil depth.

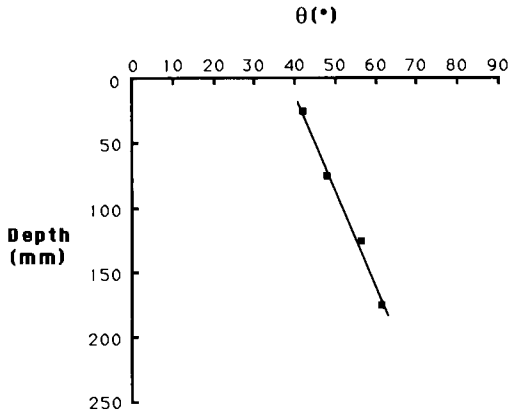


Fig. 8. Changes in the mean angle, θ° , made between the burrow and the horizontal with depth, in mm.

$$\theta = 35.20 + 0.13z \quad R^2 = 0.99 \quad (1)$$

The boundary conditions are $z > 0$ mm (since burrows cannot occur above the soil surface) and $z < 410$ mm (since burrows cannot exceed 90° from the horizontal). At this site Barley (1959) noted that burrows in the top 150 mm were irregular but that at greater depths they seldom branched and that they had the form of nearly vertical cylinders.

Plant roots growing vertically in this soil are more likely to encounter burrows near the soil surface as this is where burrows are nearest to horizontal. Dexter (1986) and Jakobsen and Dexter (1988) have illustrated the importance of vertical biopores for root growth, particularly for penetration of compacted soil. Dexter and Hewitt (1978) have shown that plant roots meeting an interface within the soil are frequently deflected. Plant roots which encounter earthworm burrows are likely to be deflected and follow a path of least resistance which will usually be downwards along the burrow.

If the additional measurement of burrow diameter was taken for each excavated segment, burrow volume could be estimated. Knowledge of the existing burrow volume at given depths together with earthworm numbers and ingestion rates may allow the longevity of burrows at various depths to be calculated by comparing the volume of burrow produced with that mapped.

CONCLUSIONS

The geometry of surface open pores is an important factor in water and air movement in soils. The techniques described in this paper show that it is possible to quantify and clearly present information on earthworm burrows using the geometrical presentations of Willoughby (1967). The technique is also

likely to be suitable for quantitatively describing burrows of other soil fauna. No correlations were found between any of the burrow parameters measured here and soil water content or soil temperature. Because the burrows found at this site had a mean length of only 392 mm and a depth not exceeding 250 mm the size of the framework used for mapping could be reduced for future measurements. Preliminary excavations at other sites would indicate appropriate dimensions for other species. Burrows created by *A. rosea* and *A. caliginosa* were not vertical near the soil surface but tended toward vertical with increasing depth. We have quantified the observation of Barley (1959) that burrows at this site seldom branched below 100 mm depth.

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Relationships between soil porosity, root development and soil enzyme activity in cultivated soils

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ABSTRACT

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Porosity and pore size distribution from thin sections, prepared from undisturbed Ap horizon samples taken from a zero- versus conventional-tillage field experiment, were analysed by means of a Quantimet 720 image analysing computer. The length and the size distribution of plant roots were determined using the same image analyzer. Soil urease and phosphatase activity in soil samples from the plots of this field experiment was also determined.

Total porosity was significantly higher in conventionally tilled plots, but the proportion of pores ranging from 30 to 500 μm in equivalent pore diameter, which are considered the most important both in soil–water–plant relationships and in the maintenance of a good soil structure, was higher in no-tilled plots. Root development showed a strict relationship with the presence of smaller pores which were more numerous in no-tilled plots. Enzyme activity was also higher in these plots than in the conventionally tilled plots.

The relationships of such enzyme activities and the various pore size classes in each type of soil showed a positive common trend between the two enzyme activities and the percentage area of pores ranging from 30 to 200 μm in equivalent pore diameter. A significant correlation was observed between the degree of porosity in this range and urease activity.

INTRODUCTION

The need to reduce the energy input in agriculture and to check the degradation of soil structure have caused farmers to consider no-till cultivation practices as an alternative to conventional tillage. Abandoning traditional farming rotations and adopting intensive monocultures, without application

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of farmyard manure to the soil, has decreased the organic matter content of Italian soils with evident deterioration of soil structure. The resulting soil porosity conditions are often unfavourable for crop growth (Pagliai et al., 1983, 1984).

The effects of soil tillage on arable land have often been related to soil physical properties, in particular soil structure, because such effects are macroscopic and easy to determine (e.g., bulk density, porosity, aggregate stability, water infiltration, etc.). There is limited information on the long-term effects on soil biology. Correlations between physical and biological soil properties are very rare.

The main objective of this paper was to investigate the long-term changes in soil microstructure and porosity after 12 years of zero and conventional tillage in a clay loam soil and their relationships with root development and soil enzyme activity.

The changes in soil microstructure were quantified through the characterization of soil porosity on thin sections of undisturbed soil by image analysis. Although the images were only analyzed in two dimensions, this method provides useful information on the complexity of pore patterns in soils not obtained by using other methods. Parameters such as pore size distribution, pore shape and relative position of aggregate and pores are very important for the evaluation of soil structure conditions in relation to root development and to properties related to soil biological activity.

MATERIALS AND METHODS

Soil and treatments

A long-term field experiment study was established on a clay loam soil (Vertic Xerofluvent) on a farm located near Bologna, Emilia in 1978. The experiment was carried out on plots planted to grapevines. Two kinds of management practices were tested: continuous no-tillage and continuous conventional tillage (tillage by a cultivator at the end of the winter to a depth of 20 cm and in summer to a depth of 10 cm for weed control). In no-tilled plots weeds were controlled with herbicides (Caragard, Ciba-Geigy). The experiment was performed with six replications. Further details were reported by Pagliai et al. (1983, 1984).

Porosity measurements

Six undisturbed samples were collected from the Ap horizon of each plot (replication) in the period of grape ripening when good soil conditions are critical for crop development. Samples were dried by acetone replacement of water (Miedema et al., 1974; Murphy, 1986), impregnated with a polyester

resin and made into 6×7 cm, vertically oriented thin sections (Murphy, 1986). For all the experiment 72 thin sections were prepared.

Photographs of the sections (Pagliai et al., 1984) were analysed by the image-analysing computer Quantimet 720 to measure total porosity, pore shape and pore size distribution. These photographs covered 4.5×5.5 cm² of the thin section, avoiding the edges where disruption could occur. Pores were measured by their shape which is expressed by the area (A) to square perimeter (P^2) ratio (A/P^2), and were divided into three shape groups: more or less rounded pores ($A/P^2 > 0.04$); irregular pores (A/P^2 0.015–0.04); elongated pores ($A/P^2 < 0.015$) (Bouma et al., 1977). Pores of each shape group were further subdivided into size classes according to the equivalent pore diameter for rounded and irregular pores and the width for elongated pores (Pagliai et al., 1983, 1984).

Root density measurements

In order to study the variation in root density, six samples for each plot and from ~ 50 cm from the trunk of grape were taken in the Ap horizon by the auger method using 5 cm diameter steel tubes (Böhm, 1979) at the same time of the sampling for the thin section preparation. To separate roots from the soil the core segments were immersed in solution of sodium pyrophosphate for 1–2 days to assist dispersion of clay. Roots were washed out of the soil in a stream of water over a 0.6 mm mesh sieve (Drew and Sakar, 1980; Bennie et al., 1987). They were then removed from the sieve and filtered into a paper filter. The samples thus collected were analysed using the Quantimet 720 (Pezzarossa and Pagliai, 1990).

The root length was determined dividing the perimeter of the roots by 2 ($P/2$). The root density was expressed in cm of visible roots per cm³ of soil. The amount of non-grape roots was negligible because weed roots are thinner and, therefore, easy to separate when washing out of the soil. The total length was further subdivided into four size classes, identified in terms of diameter. This parameter was calculated from the area and perimeter value of the roots, following the same procedure used for the determination of the width of elongated pores in the soil thin sections (Pagliai, 1983; Pagliai et al., 1984).

Soil enzyme activity

In the areas adjacent to the sampling for thin section preparation and root density measurements at the same time, additional soil samples (six for each plot) were taken for the evaluation of soil enzyme activities such as urease and phosphatase, which have shown a relationship with microbial growth and activity in soils (Frankenberger and Dick, 1983). Urease and phosphatase

activities were determined according to the methods described by Ceccanti et al. (1978) and Nannipieri et al. (1978), respectively.

RESULTS AND DISCUSSION

Porosity

The total porosity formed by pores greater than 30 μm was significantly higher in samples of conventionally tilled plots than in those of no-tilled plots (Table 1), confirming previously reported findings (Pagliai et al., 1983, 1984; Pagliai, 1987). The elongated pores represented the dominant fraction of soil porosity, particularly in samples of conventionally tilled plots.

For a thorough characterization of soil pores, the main aspects to be considered are pore shape and pore size distribution, because these are related to many important plant and soil processes such as ease of root penetration, storage and movement of water and gases. Pores ranging from 0.5 to 50 μm in equivalent pore diameter are the storage pores, while pores ranging from 50 to 500 μm are the transmission pores (Greenland, 1977) and pores ranging from 100 to 200 μm are used by feeding roots to grow into (Russell, 1978).

Pore shape and size distributions of samples of no-tilled plots showed strong differences with respect to those of samples of conventionally tilled plots (Fig. 1). The higher porosity found in the latter plots was due to the proportion of elongated pores larger than 500 μm but the proportion of elongated pores in the range of transmission pores was higher in the no-tilled plots. The increase of these kinds of pores indicates an improvement of soil structure. In fact, it has been found that a decrease in soil structure can be related to decreases in the proportion of pore space present in transmission and storage pores (Greenland, 1981). The larger elongated pores in no-tilled plots were mostly biopores which are very important for water movement (Bouma, 1982; Ed-

TABLE 1

Effects of two types of soil management on soil porosity expressed as a percentage of the total area occupied by pores per thin section (mean $\pm$ 1 SE); means of six replicates (36 thin sections: 6 thin sections $\times$ 6 replicates)

Type of pores	No-tillage	Conventional tillage
Rounded pores	3.1 $\pm$ 0.4b	1.6 $\pm$ 0.3a
Irregular pores	5.4 $\pm$ 0.6a	3.8 $\pm$ 0.4a
Elongated pores	11.2 $\pm$ 1.6a	22.6 $\pm$ 1.9b
Total porosity (%)	19.7 $\pm$ 1.6a	28.0 $\pm$ 2.1b

A different lower case letter within a row indicates a statistically significant difference at $P < 0.05$ level employing Duncan's Multiple Range Test.

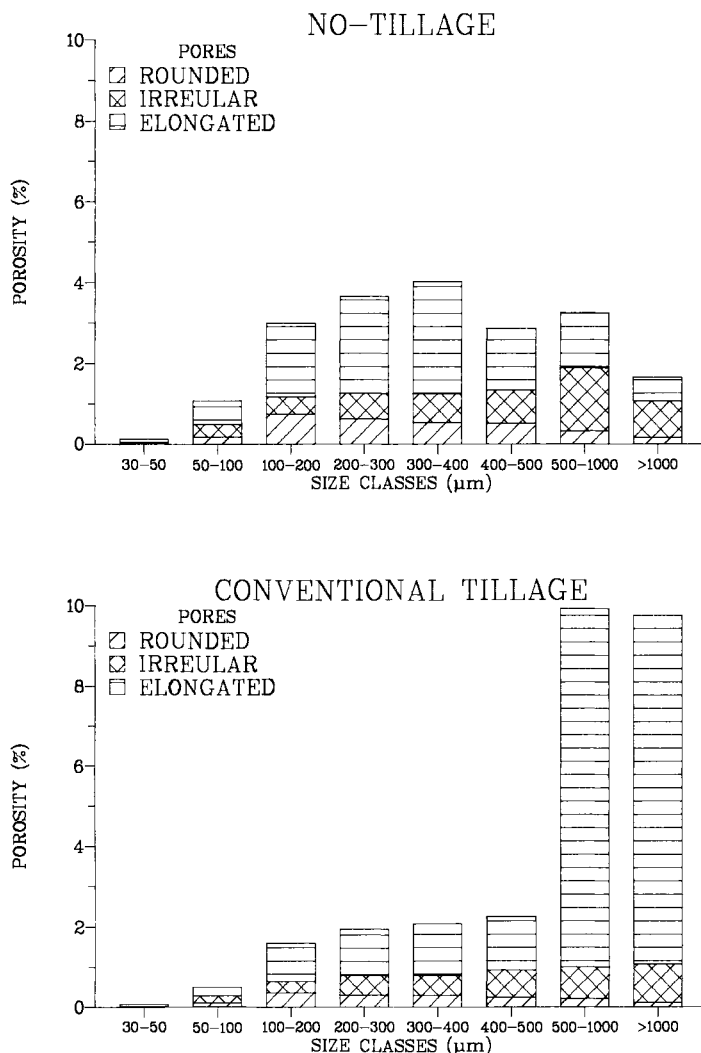


Fig. 1. Pore size distribution, according to the equivalent pore diameter for regular and irregular pores and according to the width for elongated pores, in the surface layer (0–10 cm) of no-tilled and conventionally tilled plots.

wards et al., 1988). The larger elongated pores in conventionally tilled plots were planar pores surrounding or separating aggregates or clods formed by tillage practices.

The differences in soil porosity between the two types of tillages were reflected in the type of soil microstructure that existed in each practice. The microscopic examination of thin sections revealed that a subangular blocky

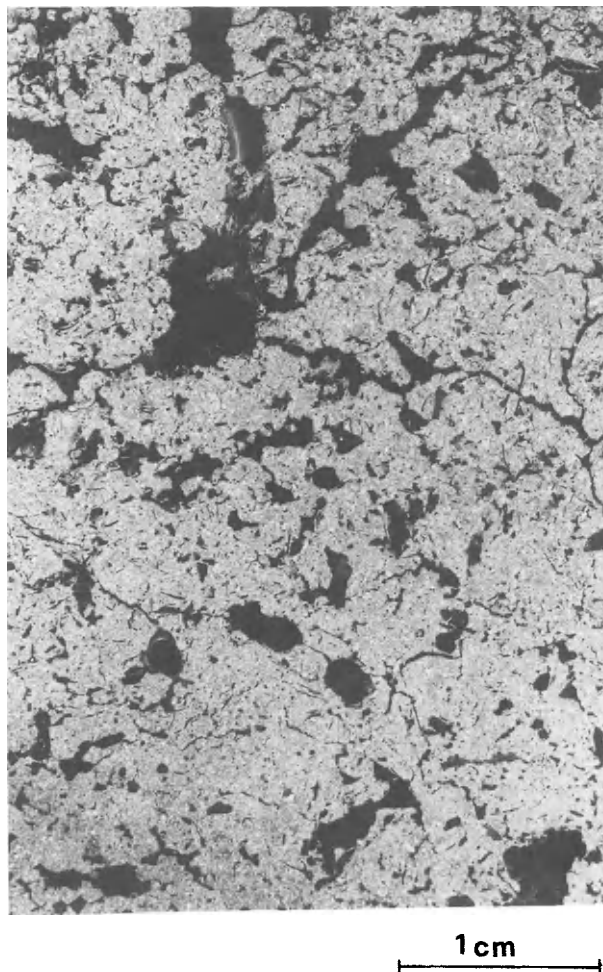


Fig. 2. Macrophotograph of vertically oriented thin section from samples of the surface layer (0–6 cm) of no-tilled plots. $2\times$. Crossed nicols.

microstructure was homogeneously present along the profile of the Ap horizon of the no-tilled plots (Fig. 2). The observation of the soil profile in the field revealed a medium to fine subangular blocky structure uniformly distributed in this horizon (Pagliai et al., 1984). In the conventionally tilled plots the microstructure was more complex: at the soil surface a platy structure was present, and resulted from soil compaction and by the formation of surface crusts (Fig. 3); below this layer in large areas a vughy microstructure (Bullock et al., 1985) was visible (i.e., numerous irregular pores break up the continuity of fine material), and other areas showed a crumbly to granular

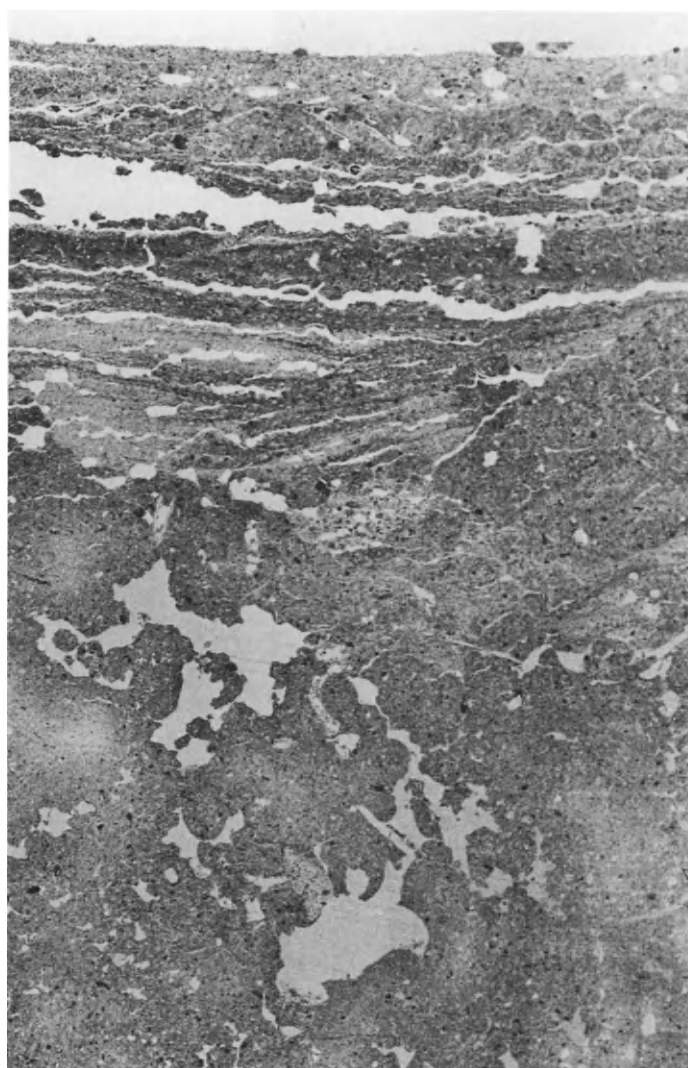
**a**

Fig. 3. Macrograph of vertically oriented thin section from samples of the surface layer (0–6 cm) of tilled plots. The presence of a surface crust is visible. $2\times$. (a) Plain light. (b, overleaf) Crossed nicols.

structure (i.e., large aggregates rather compact inside and separated by large planar pores) (Fig. 4).

The microscopic examination also revealed a lower quantity of faecal pellets, mainly represented by mammillated earthworm excrements, in samples of conventionally tilled plots as compared to those of no-tilled plots, suggesting a greater soil fauna activity in no-tilled plots.

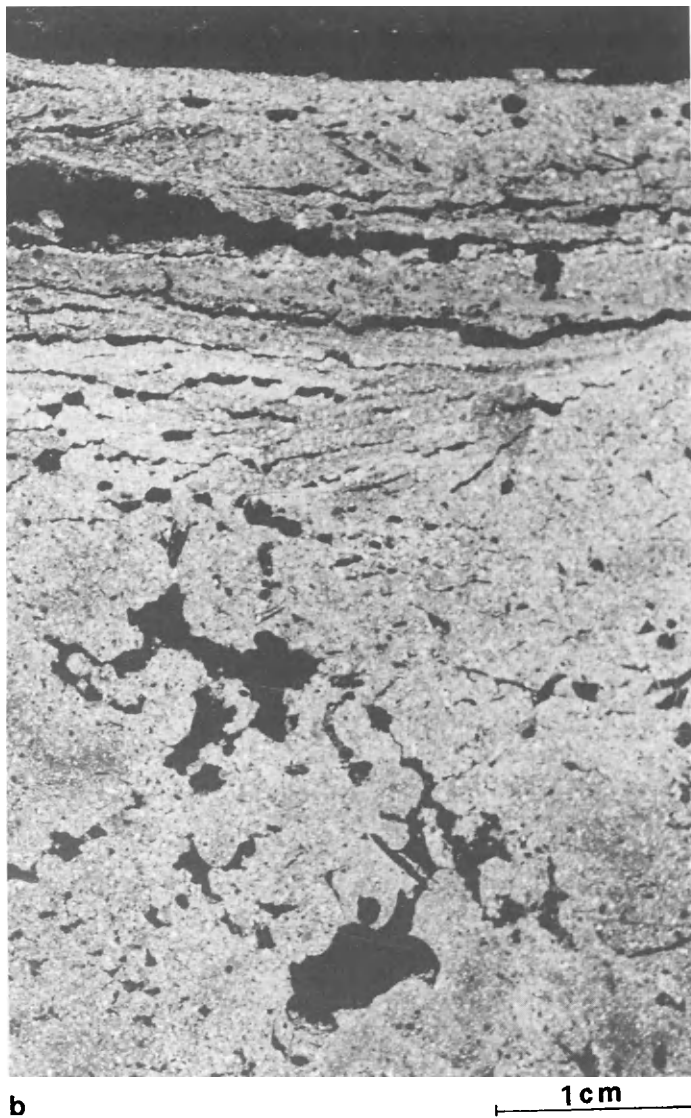


Fig. 3. continued.

Root density

The distribution of the roots in the Ap horizon (Fig. 5) showed a higher root density in the no-tilled plots than in those which were conventionally tilled.

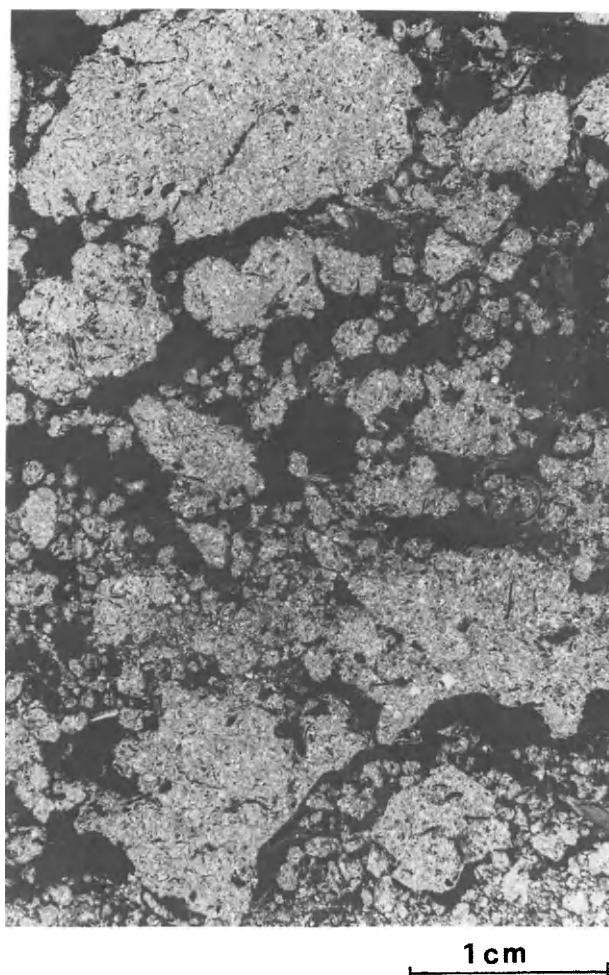


Fig. 4. Macrophotograph of vertically oriented thin section from samples of the layer 5–10 cm of tilled plots. $2\times$. Crossed nicols.

The highest proportion of the root system was represented by roots with a diameter $< 500\ \mu\text{m}$. In these size classes (0–0.5 mm) the root density was significantly higher in no-tilled plots, and followed the trend of the elongated pores. The amount of roots larger than $500\ \mu\text{m}$ tended to be higher, but not significantly different, in the no-tilled plots. This is despite the quantity of larger ($> 500\ \mu\text{m}$) elongated pores which was higher in conventionally tilled plots. These findings confirmed the importance of presence of smaller pores for root development. Thus for the soil examined in this investigation, mini-

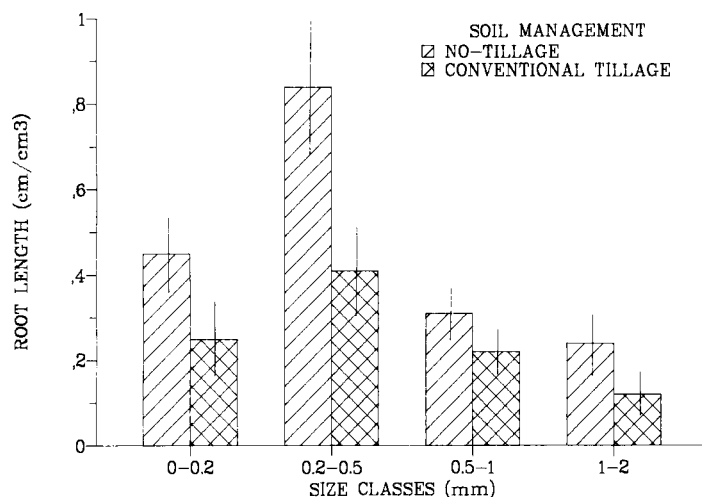


Fig. 5. Root density expressed as root length/cm³ of soil and the size distribution of the roots according to diameter. Bars represent the confidence interval at the level of 5%.

mum and no-tillage systems seemed to be more appropriate in maintaining favourable soil porosity by preserving the elongated transmission pores which allow for good root development.

Microscopic observations confirmed that root remains were more abundant in thin sections of no-tilled plots (Fig. 6) than in those of conventionally tilled plots. This because in no-tilled plots there was more roots present. Moreover, the decay of dead roots was slower in no-tilled plots.

Soil enzyme activity

Enzyme activity in no-tilled plots was higher than in conventionally tilled plots (Fig. 7). This was probably caused by the less heterogeneous soil structure of no-tilled plots. Similar results were obtained by Dick (1984) who reported that the activity of various enzymes were greatly amplified in the soil surface of no-tilled soils.

Urease activity was positively correlated with soil porosity ranging from 30 to 200 μm (Fig. 8). This suggests that the presence of pores in this size range is important for both root development and soil enzyme activity. Phosphatase activity was not significantly correlated with soil porosity ranging from 30 to 200 μm . This suggested that the urease activity is more closely related to the quantity and quality of soil porosity, than is phosphatase activity (Frankenberger and Dick, 1983; Sequi et al., 1985).



Fig. 6. Microphotograph of vertically oriented thin section from the surface layer (0–6 cm) of no-tilled plots. Root remains in the small elongated pores with rather regular walls are visible. In these plots the surface crust was practically absent and the continuity of elongated pores was not interrupted at the soil surface. The presence of faecal pellets can also be noticed. 25 $\times$. Plain light.

Information is limited on the distribution of roots and soil organisms in the soil pores, and information on the distribution of soil enzymes is completely lacking. The capillary pores may represent the most favourable microhabitats for bacteria, and some protozoa feeding on bacteria may be able to move in such pores. This assumes a mean diameter of 5 μm for flagellates, 10 μm for amoebae and 20 μm for ciliates. However, it seems reasonable to assume that pore sizes favourable to the growth of living roots, ranging from 30 to 200 μm , provide nutrients and carbon sources for living organisms, while smaller pores are explored by root hairs.

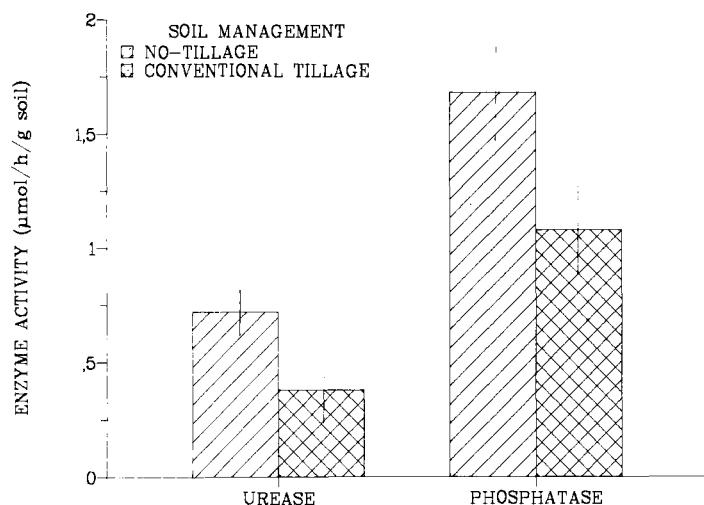


Fig. 7. Urease (left) and phosphatase (right) activities in the surface layer (0-10 cm) of no-tilled and conventionally tilled plots. Bars represent the confidence interval at the level of 5%.

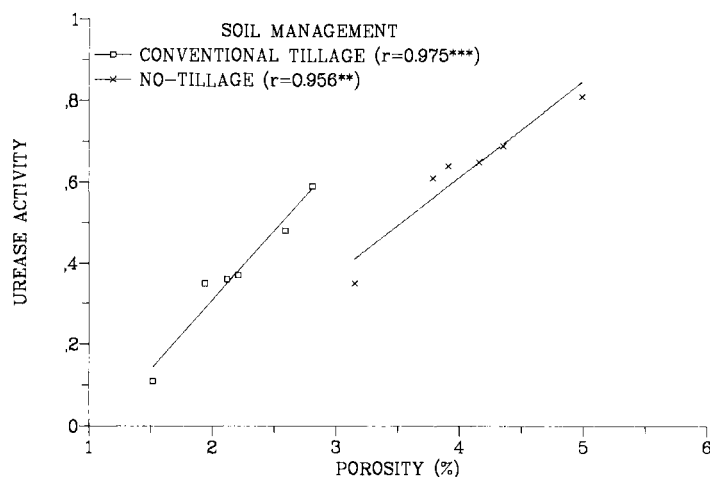


Fig. 8. Positive correlation between urease activity ($\mu\text{mol ammonium release h}^{-1} \text{g}^{-1} \text{soil}$) and the porosity in the range of 30-200 μm in no-tilled and conventionally tilled plots.

CONCLUSIONS

The results of this study confirmed that the intensive long-term conventional tillage practices caused damage to soil structure. The reduction of transmission pores and the increase of the largest pores, with respect to no-tilled plots, produced large soil aggregates which were rather compact inside and less stable to physical stresses caused by traffic compaction, raindrop im-

pact, etc., as indicated by the presence of a platy structure. The pore pattern of the conventionally tilled plots also decreased the suitability of the soil as a rooting environment for crops.

These results clearly showed that the rooting potential of no-tilled soil was better because of a greater proportion of transmission pores. Transmission pores, which are the most important for good soil structure and for water movement in soil, were positively correlated with root growth and with soil enzyme activity.

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Semi-automatic image analysis of earthworm activity in 2D soil sections

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ABSTRACT

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A method is presented for quantifying various morphological changes of the soil fabric caused by earthworm activity. Adult earthworms were kept in planar glass cuvettes for 27 days (in one case for 45 days). The investigations were conducted with the endogeic species *Aporrectodea caliginosa* and the anecic species *Aporrectodea longa*. The distance between the glass sheets was suited to the diameter of the earthworms. This artificial, two-dimensional profile made possible the observation of the complete burrow system. The cuvettes were filled with sieved soil (aggregates of 1 to 2 mm diameter) so that morphological changes of the soil fabric could easily be detected. Photographs were taken of both sides of the cuvettes every 3 days forming a chronological series. For cast production and burrow system studies the photographs were analysed semi-automatically on a digitizing tablet with a morphometric programme. Cast production and total burrow length were greater in *A. caliginosa* compared to *A. longa* for the same period of time. Continuity of the burrows was also higher in *A. caliginosa* (60–70% of the total burrow length) than in *A. longa* (40–50%). In respect to the degree of reticulation of the burrows no difference between the two species was found.

INTRODUCTION

One of the main difficulties in researching the mutual relationships between organisms and soil structure lies in the opacity of the subterranean environment. Nevertheless, it is possible to investigate complete earthworm burrow systems in the soil by cutting soil cores into slices in order to assess size and location of burrow fragments (Rushton, 1986; Wendt and Larink, 1990), by X-ray computed tomography (Joschko et al., 1991) and by planar cuvette technique (Evans, 1947). The last method is a simple and effective way of visualizing processes along earthworm burrows, such as water move-

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ment (Schrader and Joschko, 1991) and changes of redox potential and pH (Schrader, 1991). So far, most quantitative investigations on earthworm cast production concerned surface deposited faeces both in the field (e.g. Graff, 1961; Elliott et al., 1991) and in laboratory experiments (e.g. Shipitalo et al., 1988; Kretzschmar, 1991).

In the present study earthworms were kept in cuvettes to quantify their soil turnover, expressed as cast production, burrow length and degree of reticulation of the burrow system. Two earthworm species with different ecological strategies were used (*sensu* Bouché, 1977): the endogeic *Aporrectodea caliginosa* (Savigny, 1826) and the anecic *Aporrectodea longa* (Ude, 1885). Kretzschmar (1990) evaluated the burrow system of *A. longa* after nearly 7 months in soil columns. He considered only the burrows on the column walls, supposing them to include nearly 90% of the whole burrow system, so that his conclusions about burrow length were based on burrow fragments.

Recently, Jastrow and Miller (1991) reviewed methods for assessing the effects of biota on soil structure. They emphasized the importance of studies showing the sequence of events involved in the development of soil structure under the influences of biota. Therefore the aim of this study was to determine the effect of earthworms burrowing in a certain period of time. Evaluation was carried out by photographic technique coupled with image analysis.

MATERIAL AND METHODS

Experimental design

The experiment was conducted in planar glass cuvettes with inside dimensions 23 cm high and 40 cm wide for *A. caliginosa* and 40 cm high and 23 cm wide for *A. longa*. A distance of 0.6 cm between the two glass sheets made it possible to observe the whole burrow system. In order to distinguish morphological changes of the soil fabric due to earthworm activity the cuvettes were filled with sieved soil aggregates (fraction 1 to 2 mm). The soil was from the Ap horizon of a Parabraunerde from loess (Luvisol). It was moistened to a gravimetric water content of 17%. The dry bulk density ranged from 1.2 to 1.4 g cm⁻³.

Before the experiment the earthworms' fresh weight was determined. Two adult earthworms (with clitellum) of one species were put on the soil surface of each cuvette. They were fed with dried and pulverized cattle manure which was placed on the soil surface. The cuvettes (two per species) were covered with laboratory film (Parafilm) and placed vertically in a dark room at 12°C for 27 days. Every 3 days photographs were taken from both sides of the cuvettes so that each photograph showed one side in total. Prints of the negatives were made showing the soil to a scale of 1:1. One cuvette with *A. caligi-*

nosa was observed for 45 days (only five photographs were taken) to observe comparatively "long-time effects".

At the end of the experiment the cuvettes were dried and all casts were collected. They could easily be separated from the soil aggregates with a spatula and needle. The weight of the collected casts was compared with the calculated cast weight.

Image analysis

Data were obtained semi-automatically by using the Sigma Scan measurement system (Jandel Scientific, Corte Madera, CA 94925) coupled with an personal computer (IBM). This system consists of the morphometric calculation programme Sigma Scan 3.90 and a tablet which digitizes objects with an accuracy of ± 0.127 mm (0.005 inch) by a pen (cursor) which is connected to the tablet.

Each photograph was placed on the tablet. Using the cursor outlines of casts and midlines of burrows, which had been marked black before, were traced by hand. The following measurement of cast areas and burrow lengths occurred automatically.

According to Bullock et al. (1985) earthworm casts are mammilated excrements, bodies with mutually interfering smooth subrounded surfaces, with macroscopically homogeneous internal fabric. Due to this characteristic structure casts could easily be distinguished from the regulary formed soil aggregates. Because the earthworms deposited the casts in contact to the glass sheets the casts could be assumed to be of approximately cylindrical rather than spherical shape with a longitudinal axis of maximal 0.6 cm due to the sheets' distance. To estimate the total volume the data of the cast areas calculated from both sides of the cuvettes were multiplied by the factor 0.3 cm (half the distance between the sheets) and added. The mass of the produced casts was calculated by multiplying the total volume by the bulk density of the casts (1.7 g cm^{-3}). The bulk density was determined by Teiwes' cement method modified by Dunker (1990).

Burrows are defined as elongated, cylindrical or arched voids with regular conformation. Continuous burrows are parts of the burrow system which are longitudinally open to one side of the cuvette. The total burrow length includes length of burrows plugged by casts and continuous burrows in sum. This item was determined from one side of the cuvettes because each side showed the whole burrow system. Whereas continuity could only be determined by considering both sides. Finally, spots where burrows cross (branching nodes) were counted to provide information about the degree of reticulation.

RESULTS

Cast production and burrow length of both species increased steadily during the whole time. The dry weight of casts produced by *A. caliginosa* was on average twice that produced by *A. longa* in the same period of time (Fig. 1).

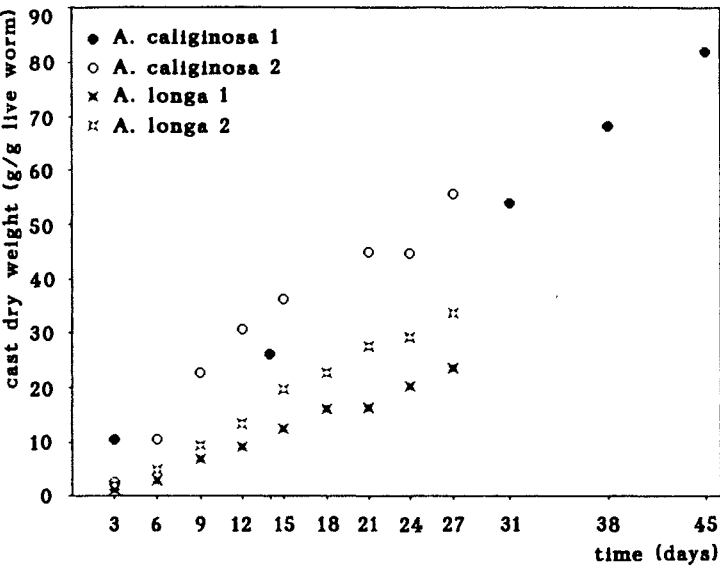


Fig. 1. Cast production of *A. caliginosa* and *A. longa*. The data of each cuvette are presented separately (1 and 2).

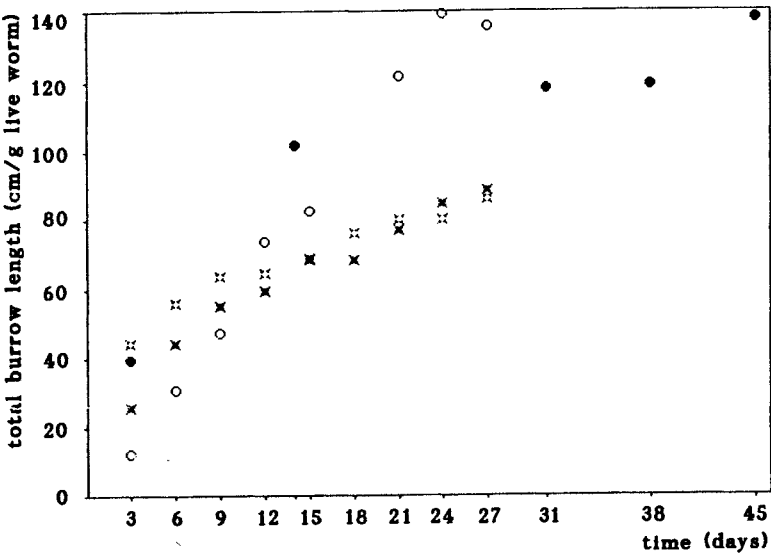


Fig. 2. Total burrow length of *A. caliginosa* and *A. longa* (for legend see Fig. 1).

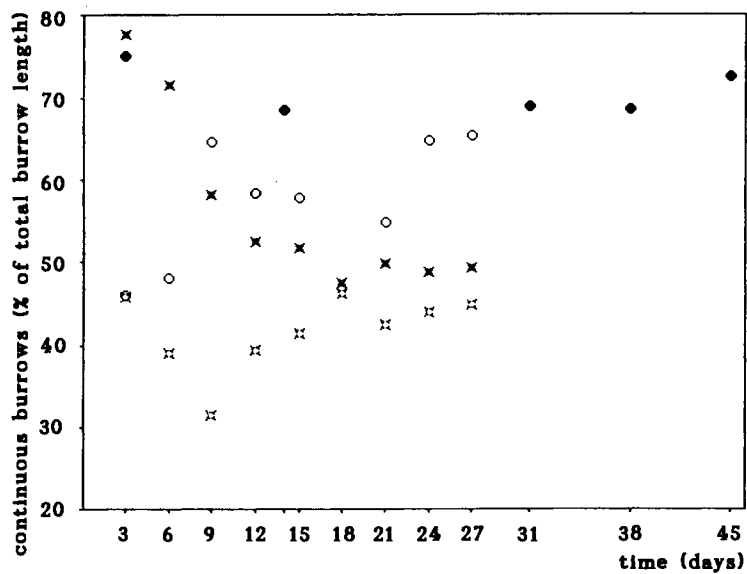


Fig. 3. Continuous burrows relative to total burrow length of *A. caliginosa* and *A. longa* (for legend see Fig. 1).

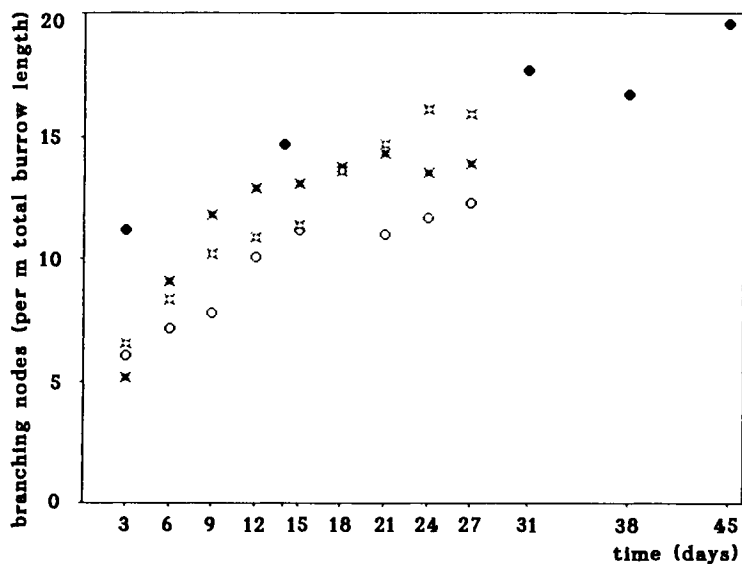


Fig. 4. Branching nodes as degree of reticulation of *A. caliginosa* and *A. longa* (for legend see Fig. 1).

The total burrow length was greater for *A. caliginosa* than for *A. longa* (Fig. 2) which was not obvious before 12 days. The length of continuous burrows, expressed as a percentage of total burrow length, varied over a wide range from ~30 to 80% during the first two weeks (Fig. 3). After this time the two earthworm species showed distinct differences. About 60–70% of the burrows of *A. caliginosa* were continuous but only 40–50% of those of *A. longa* were continuous. The number of branching nodes did not differ between the two species (Fig. 4).

DISCUSSION

The method presented is suitable for quantifying, chronologically and thoroughly, possible morphological influences of earthworms on the soil fabric. The two-dimensional form of the artificial experimental environment was obviously without disadvantage to the earthworms since the live weight remained the same on average, before and after the experiment. This is similar to Martin (1982) who detected a 2D system not disturbing to earthworms.

Earthworms exude soil in the form of a saturated paste (Barley, 1959). So their casts were well contrasted to the surrounding homogeneously aggregated soil. It was impossible to determine automatically the areas of casts by grey level information as the casts and the soil had the same grey level. In this situation the perceptive faculty of a trained observer combined with semi-automatic image analysis was required (Maier-Kühne, 1986) in order to assess quantitatively cast production. In comparison with the measured values of cast weight the calculated values were lower at the end of the experiment. But the accuracy of the method was satisfactory as the difference was below 10%. Dry bulk density of casts was higher than the soil. This occurs in loose soil when $d_B \leq 1.4 \text{ g cm}^{-3}$ (Söchtig, 1992).

The data presented in Figs. 1 and 2 correlate with the endogeic way of life of *A. caliginosa*. This species ingests and egests much more soil than anecic earthworms do (Lavelle et al., 1989). The finding that more burrows of *A. caliginosa* were continuous in respect to total burrow length was surprising because, according to the literature, endogeic earthworms deposit most of their casts below ground (e.g. Lavelle et al., 1989). Separating the weighed casts at the end of the experiment into those from above and those from below 10 cm depth showed that *A. longa* egested ~60% of its casts at a depth below 10 cm, while *A. caliginosa* egested only 30% below this depth. The burrow systems of both species studied were dynamic in the sense of continuity. Most of the burrows were semi-permanent, that is, they were more than once plugged by casts and burrowed again.

According to Bouché's ecological classification of earthworms (1977) *A. longa* is anecic in contrast to the endogeic *A. caliginosa*. Therefore the cuvettes had a different proportion for each species. Although the experiment

was carried out in an artificial environment it can be concluded that *A. longa* is rather endogeic than anecic. Also Kretzschmar (1991) termed *A. longa* endogeic. These results suggest that life form of *A. longa* should be studied more closely.

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Synlocation of biological activity, roots, cracks and recent organic inputs in a sugar beet field ^α

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ABSTRACT

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Soil tillage does not lead to homogeneously mixed soil layers. Depending on the interaction between the physical condition of the soil and the tillage implement, a repetitive, layered pattern of accumulated crop residues or other freshly added organic matter can often be observed. Such a non-homogeneous pattern may affect subsequent biological activity, the rates of decomposition, and the chances for root development to occur close to the sites of mineralization in the next growing season.

Patches of organic matter, soil cracks and roots were mapped on clear plastic (polythene) sheets on a soil profile wall. Maps were analysed for spatial correlation of the various mapped items. Small samples of the patches of crop residues and freshly added organic matter, and of surrounding (control) soil were analysed for the abundances of bacteria and protozoa.

A statistical test for synlocation of roots and cracks was developed, on the basis of measured root densities as a function of the distance to the nearest crack. A significant increase of root density (by a factor two) close to the crack was found. For organic matter patches a similar synlocation with cracks was indicated.

Patches with freshly added organic matter contained twice as many bacteria and five times as many protozoa as the control soil. Patches with recent crop residues contained four times as many protozoa, but equal densities of bacteria as control soil. Only a small part of the spatial variability in the abundance of soil organisms, encountered when using a random sampling scheme, is accounted for by distinguishing these patches. Nevertheless, knowledge of the location and activity of these "hot spots" may help in understanding the process of decomposition. The present data indicate a certain degree of synlocation of roots and sites of increased n mineralization, but its effect on plant nutrition and on N use efficiency is probably small.

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INTRODUCTION

Restricted accessibility of resources to organisms, even when located close together, is a focus of interest in soil ecology. The structure of the soil largely determines the accessibility of food to organisms, and of organisms to their predators. Most attention in this respect has been directed at the accessibility of fine pores, as determined by the body size (diameter) of soil organisms. However, differences in accessibility might exist also at a larger scale. Soil organisms differ widely in their mobility and "home range", and therefore differ in the scale of the patchiness of resource supply to which they can respond, either as individuals or as populations. Colonization of and ecological succession on recent organic inputs by soil organisms, leading to decomposition and mineralization, may thus depend on the location and distribution of such inputs in the soil.

Soil tillage does not lead to a homogeneously mixed plough layer. Rather, depending on the interaction between the physical condition of the soil and the tillage implement, a layered pattern may develop, with accumulations of crop residues or other freshly added organic matter in a repetitive pattern. Such a non-homogeneous pattern has the potential to affect the subsequent rate of decomposition through effects on biological activity, and may also affect the chances that roots occur close to the sites of mineralization in the next growing season. Quantification of such effects is needed.

Branch root development may also respond to local patches of mineral nutrients (Wiersum, 1958; Drew and Saker, 1975; De Jager, 1982), of organic acids (Wiersum, 1974) or of loose soil around cracks. If roots were located preferentially close to recent organic inputs (synlocation) they would benefit more directly from N-mineralization. A statistical test of spatial correlation of roots with patches of organic matter, or with cracks can be based on a comparison of point densities (number per unit surface area) at increasing distances from the objects of interest (Fig. 1). Such comparison can be made with computer image analysis methods (Van Noordwijk et al., 1993), where the area within a certain distance of the crack can be identified and measured, as well as the number of roots for each distance class.

Although heterogeneous distribution of roots and soil organisms is relevant in its own right, it also contributes to "sampling error", if average densities are to be measured. The coefficient of variation for root weight and root length per sample of 385 cm³ is normally between 0.3 and 0.5 for relatively homogeneous agricultural soils (Van Noordwijk et al., 1985). Sampling schemes acknowledging different positions in row crops, especially in the top layers and in young plants, may help to reduce sampling error (Van Noordwijk et al., 1985). At the start of the Dutch Programme on Soil Ecology of Arable Farming Systems, similar sampling schemes were devised for the various types of soil organisms, in the expectation that at least some organisms might be

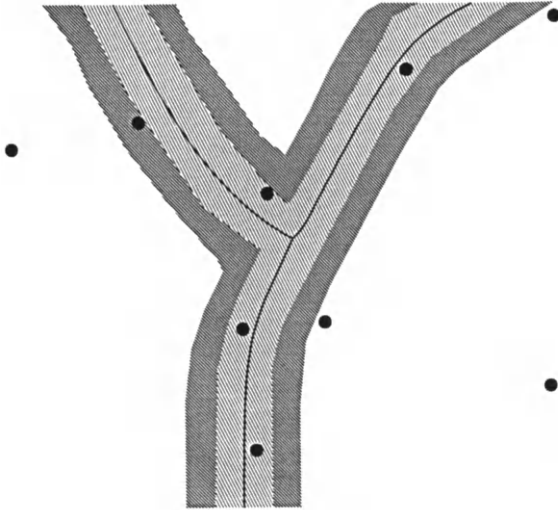


Fig. 1. Tests of spatial correlation of roots (●) and cracks (lines or irregularly shaped area) can be based on a classification of the map area by distance to the cracks and measurement of both surface area and number of points for each distance class.

spatially correlated with roots (Brussaard et al., 1990). Bacteria and protozoa appeared to be normally distributed in the soil with a coefficient of variation per composite sample of ten 100 cm³ subsamples of ~10% (Bloem et al. 1992a; Zwart, unpublished). We expected that at a smaller scale, densities of soil organisms might correlate with concentrations of recent organic inputs. Moreover, the distribution of patches of organic inputs might be related to the tillage system (Staricka et al., 1991) rather than being randomly distributed in the plough layer. If locally high densities were to occur in macroscopically identifiable patches of organic matter, sampling schemes should account for this type of spatial variation.

We tested whether biological activity differed between macroscopically identifiable subsamples of top soil, representing (i) the bulk soil, (ii) recognizable crop residues and (iii) a recently incorporated, organic amendment. Spatial correlations of cracks, organic matter and roots were tested on the basis of maps made of a transect of the plough layer.

METHODS

The test site is located at the Lovinkhoeve Experimental Farm in the Noordoostpolder (Marknesse, The Netherlands). The soil is a calcareous silt loam of pH (KCl) 7.5, and was reclaimed in 1942. Annual precipitation at the site is usually between 600 and 950 mm.

The results presented in this paper are from two management practices:

integrated and conventional. Integrated differs from the conventional practice in that (i) N-fertilizer application rates are reduced to 50–65% of those recommended in conventional practice (viz. from 130–285 to 65–170 kg per ha per year, depending on crop), (ii) pesticide application is reduced, as is (iii) depth of soil tillage (20–25 cm plough in conventional, 12–15 cm cultivator in integrated). Due to a different soil management in the past, the organic matter content of the upper 20 cm of the conventional plot is 2.1% while that of the integrated plot it is 2.7%.

Since 1985, the four-year crop rotation on both plots has been: winter wheat, sugar beets, barley and potatoes. The data presented here are from the 1989 sugar beet crop. In the soil profile stubble and straw residues of the winter wheat, harvested in August 1988, could still be identified. Their original C/N ratio was ~ 120 . In the autumn of 1988 an organic waste product of commercial mushroom production, “champost”, had been applied to the integrated system, before soil tillage, at a rate of 30 t/ha. This product had a dry matter content of 35% and on a dry weight basis it had a C concentration of 28%, a total N concentration of 2.0% and thus a C/N ratio of 14.

The distribution of crop residues and organic matter accumulations (“champost” and others) relative to cracks and gaps was mapped on clear plastic (polythene sheets), from within a trench made perpendicular to the tractor orientation during soil tillage operations. In September 1989, root distribution in the mature sugar beet crop was mapped on polythene sheets; cracks and organic matter accumulations were mapped as well.

Maps were digitized and root density was measured as a function of distance to cracks (Van Noordwijk et al., 1993), using a Quantimet 570 image analyzer. As a statistical test of spatial correlation, a model was fitted of the form:

$$N_D = A_D [c + \exp(b_0 + b_1 \times D)]$$

where N_D is number of roots, A_D is sample area, both as a function of distance, D , the coefficient c represents the average point density not influenced by distance to the crack, b_0 determines the point density at distance 0, and b_1 the rate of change with distance. The best fit of this model, assuming that point densities have a Poisson distribution, was derived using a Genstat 5 (Payne et al., 1987). If b_1 differs from 0 in a two-sided test, we may conclude that roots and cracks are spatially correlated. A negative value for b_1 indicates a positive correlation (synlocation), a positive value indicates a negative correlation (avoidance).

On three sampling dates (April 14, May 30, June 8, 1989) small samples were carefully taken from patches which visually contained either high concentrations of (i) fine organic material (on the integrated plot this was mostly “champost”) or (ii) high concentrations of plant residues (recognizable plant structures, mostly wheat stubble and straw). As a control, samples were taken

from the surrounding soil. In the laboratory, the soil samples were analyzed for numbers of bacteria and protozoa. Bacterial numbers were determined by epifluorescence microscopic counts in soil smears after staining with Europium chelate, as described by Bloem et al. (1992b). Protozoa were determined using the Most Probable Number technique (Darbyshire et al., 1974). An index for bacterial activity was obtained by counting the frequency of dividing-divided cells [FDDC; for method see Davis and Sieburth (1984) and Bloem et al. (1992a, b)].

RESULTS

Root distribution profile maps

Figure 2 shows a map of a profile wall, sampled in six adjacent "windows" in May. In the pattern of major cracks the effect can be recognized of ploughing, with a plough blade of about 40 cm. Organic matter patches appear to be associated with these cracks, but their number is too low for a statistical test. Figure 3 gives part of a root map for the plough layer in September on the same field. Figure 4 shows root densities and occurrence of patches of recent organic matter as a function of distance to the nearest crack for the map of Fig. 3. For the roots, an acceptable fit was obtained with the model:

$$N_D = A_D [0.00058 + \exp(-6.6 - 0.10D)]$$

where the three parameters are all significantly different from 0. Distance D is measured in cm and roots were represented as a single pixel on a map where one pixel is about 0.5 mm in reality. For the organic matter patches a similar, or slightly stronger, decrease of point density with distance to the crack was found as for the roots, but the statistical model is not applicable here.

Biological activity

On the sampling dates in May and June the crop residue samples had a C content of about 28%, indicating considerable admixture of soil particles (due to internal slaking of the soil?). The C/N ratio of the retrieved residues was about 30:1, which was considerably less than that of the material worked under in Autumn 1988.

Analysis of variance showed no significant interaction between the factors place and time, so only main effects are discussed here. The factor place had a significant effect ($p < 0.01$) on the densities of protozoa and bacteria. In the stubble patches, densities of bacteria and protozoa (number per g dry soil) were higher than in the control soil (for bacteria a factor of 2; for protozoa a factor of 5; Table 1). In patches with high concentrations of organic matter, the number of protozoa was significantly higher than in the control soil (a factor of 4), whereas the numbers of bacteria were not significantly

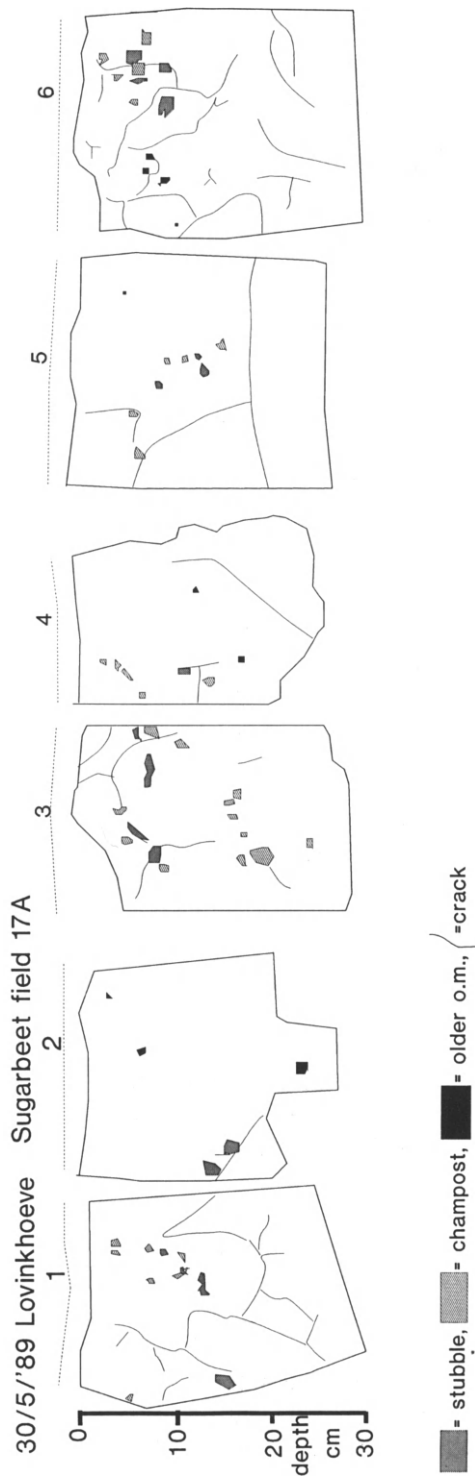


Fig. 2. Map of cracks (lines) and patches of recent organic inputs, macroscopically identifiable crop residue and older organic matter patches on vertical profile walls of the plough layer in a sugar beet field, May 30, 1989; the maps were drawn 1:1 scale on transparent plastic sheets.

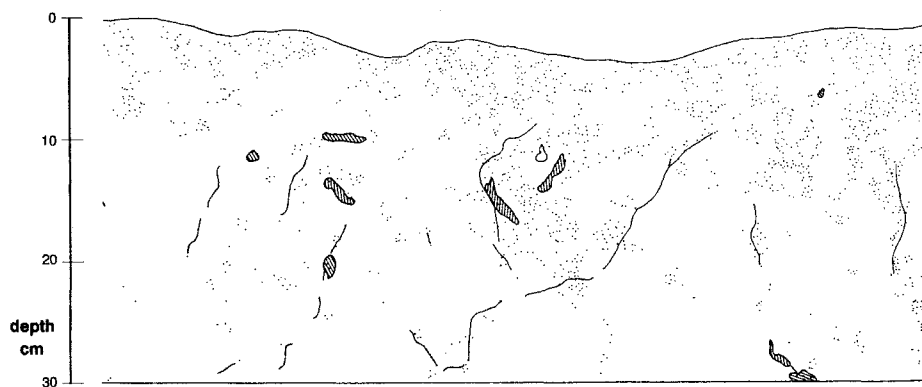


Fig. 3. Map of cracks (lines), roots (dots) and patches of recent organic inputs in the same sugar beet field as Fig. 2, on September 5, 1989.

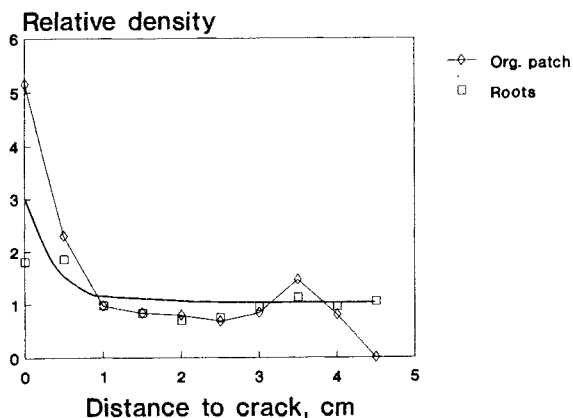


Fig. 4. Root density and occurrence of patches of recent organic inputs as a function of distance to large cracks in the profile, based on the map of Fig. 3; average point density was used to scale the results.

different. Relative growth rates of bacteria, expressed as the frequency of dividing cells (FDDC), did not differ significantly between the different types of soil samples. Bacterial density was significantly higher in May than in April and June.

Decomposition rates of organic matter (i.e. feeding rates of bacteria in $\mu\text{g C day}^{-1} \text{g}^{-1} \text{soil}$) in the different types of soil samples were calculated assuming steady-state conditions (Hunt et al., 1987), i.e., feeding rates were assumed to balance energy losses due to natural death and predation. The feeding rate of bacteria (F_{bact}) becomes:

$$F_{\text{bact}} = \frac{D_{\text{bact}} B_{\text{bact}} + P_{\text{bact}}}{e_{\text{bact}}} \quad (1)$$

where D_{bact} is the specific natural death rate (day^{-1}), B_{bact} is the bacterial biomass ($\mu\text{g C g}^{-1}$ soil), P_{bact} is the death rate due to predation, e.g., by protozoa ($\mu\text{g C day}^{-1} \text{ g}^{-1}$ soil), and e_{bact} is the production:consumption ratio of bacteria.

The predation rate by protozoa can be calculated:

$$P_{\text{bact}} = F_{\text{prot}} = \frac{D_{\text{bact}} B_{\text{bact}}}{e_{\text{prot}}} \quad (2)$$

Taking a specific death rate of protozoa (D_{prot}) of 0.02 day^{-1} (Hunt et al., 1987), and a production efficiency (e_{prot}) of 0.4 (Zwart and Darbyshire, 1992), the feeding rate of protozoa becomes 0.685, 0.515 and $0.125 \mu\text{g C day}^{-1} \text{ g}^{-1}$ in soil organic matter patches, in plant residue patches, and in control soil, respectively. Taking a natural death rate of bacteria (D_{bact}) of 0.002 day^{-1} and a production efficiency (e_{bact}) of 0.30 (for references see De Ruiter et al., in prep), the feeding rates of bacteria become 2.5, 1.8 and $0.5 \mu\text{g C day}^{-1} \text{ g}^{-1}$ soil in organic matter patches, in plant residues patches, and control soil, respectively. This indicates that, as compared to the control bulk soil, the potential decomposition rate in organic matter patches is five times higher, and in patches with plant residues it is three times higher. Following this method of calculation, the specific growth rates of bacteria [nominator eq. (1) per bacterial biomass] will amount to 0.08, 0.10 and 0.03 day^{-1} for organic matter patches, plant residues patches, and control soil, respectively. These differences in specific growth rates are, however, not confirmed by the FDDC values.

TABLE 1

Bacterial and protozoan biomass and frequency of dividing-divided cells (FDDC) in patches with high concentrations of organic matter (o.m.), high concentration of crop residues (stubble) and control soil

	Place				Time			
	<i>p</i>	O.M.	Stubble	Control	<i>p</i>	April	May	June
<i>Bacterial</i>								
No. ($\times 10^9/\text{g soil}$)	0.002	1.04 ^a	0.55 ^b	0.49 ^b	<0.001	0.56 ^b	1.09 ^a	0.44 ^b
Biomass ($\mu\text{g C/g soil}$)	0.002	33.4 ^a	17.6 ^b	15.7 ^b	<0.001	18.0 ^b	34.8 ^a	13.9 ^b
FDDC (%)	0.82	7.8	7.2	8.4	0.01	6.6 ^{ab}	11.8 ^a	4.9 ^b
<i>Protozoan</i>								
No. ($\times 10^6/\text{g soil}$)	<0.001	0.083 ^a	0.063 ^a	0.017 ^b	0.06	0.097	0.040	0.027
Biomass ($\mu\text{g C/g soil}$)	<0.001	13.4 ^a	10.27 ^a	2.48 ^b	0.06	15.0	6.86	4.34

Mean values and *p*-values are given for the factors place and time (sampling date), as no significant interactions between these factors were found; analysis of variance was based on the logarithm of densities. Any difference between values followed by the same letter is not statistically significant.

DISCUSSION

The differences in biomass of bacteria and protozoa between the different types of soil samples indicate strongly that biological processes occur at different rates in the different patches. However, the estimated decomposition rates, based on the biomass data, were not consistent with the FDDC values. The FDDC is an index of the specific growth rate (μ). Thus, although the specific growth rate seems to be similar in control soil and in organic matter, in the latter the cell production ($\mu \times \text{numbers}$) will be larger due to the higher cell numbers.

The fact that in patches with high concentrations of organic matter, the biomasses of bacteria and protozoa per unit sample weight were relatively high, indicated that the availability of food is an important environmental factor regulating their densities, especially those of the protozoa. Maps of the soil profile indicated that the patches of recently applied organic matter and plant residue were few in number and small in size. The biomasses of bacteria and protozoa were relatively high in these patches, but only by factors of ~ 2 and 4 for bacteria and protozoa, respectively. Kanazawa and Filip (1986) reported much greater differences (by factors of 10 to 100) in bacterial numbers between organic fractions and mineral soil particles fractionated from an arable brown soil by wet sieving. They used dilution plate counts and found 10^7 – 10^9 bacteria g^{-1} dry wt. in organic particles, about 10^6 bacteria g^{-1} dry wt in mineral particles of sizes between 0.05 and 0.5 mm, and about 10^7 bacteria g^{-1} dry wt. in the silt–clay fraction < 0.050 mm. The greater differences in their study, compared to our present data, may have been caused by the use of dilution plate counts for enumeration of bacteria, whereas we used direct microscopic counts. Because the results of plate counts depend on the growth medium used, they are selective and do not represent total bacterial biomass (Gray, 1990). Nevertheless, differences in plate counts between organic and mineral soil particles may indicate qualitative differences between bacterial populations which are not revealed by microscopic counts of total numbers. Another reason for the smaller differences in bacterial numbers between bulk soil and organic particles in our study may be the soil type. Kanazawa and Filip (1986) found about ten times more bacteria in the silt clay fraction < 0.050 mm than in the coarser fractions of mineral particles. Since in our silty loam soil more than 70% (v/v) of the soil particles are smaller than 0.050 mm (Kooistra et al., 1989) smaller differences in bacterial numbers between bulk soil and organic particles may be expected than in sandy soils.

It must be noted that the two- and four-fold higher numbers of bacteria and protozoa per gram of organic matter may be caused by a lower bulk density, and thus a larger volume per gram, compared to control soil. The difference in organism density may well have disappeared if sample volume rather than

sample weight had been used as the basis of comparison. No reliable estimates of bulk densities exist for the sampled patches to allow expression of organism densities in this way. As sample weight is the normal basis of reference, the results obtained suggest that for protozoa some improvement of sampling efficiency might be obtained from analyzing the stratum, "recent organic inputs", separately from the bulk soil. In a small part of the soil (roughly 2–5% of the soil volume) biological activity is about five times higher than in the "background" soil. This might be a basis for using a "stratified" sampling scheme, but a quantitative analysis of the expected gains of applying such a scheme is desirable. Cochran (1963) gave three situations when stratified sampling will produce large gains in precision: (1) strata differ in frequency; (2) strata differ in mean value; and (3) a good estimate of stratum frequency is available. The possible advantage of stratified sampling consists of a more efficient use of a limited sampling capacity (smaller variance for the estimated mean), especially if the strata differ in internal variability. For unlimited sampling intensity no advantage will be obtained; in fact, stratified sampling carries the danger of introducing a bias in the results if an unreliable estimate of the weights of the various strata is used. If no previous knowledge on strata frequencies (in our case: the volume frequency of "hot spots" in the soil) is available, stratification is only useful if a simple and reliable estimation procedure is available. It may be questioned whether the present method of mapping "hot spots" on a profile wall meets this criterion. The option of pooling (a large number of) subsamples before analysis, which does not exist in a sociological survey, and a situation where analyzing rather than taking samples is a rate limiting step, is more attractive than stratification if we want an efficient (in terms of reliability per unit effort) estimate of the mean. For a better understanding of the processes of decomposition, however, stratification may be valuable.

The differences in flow rates, assuming steady-state conditions, were of the same order of magnitude as the differences in protozoan densities. The small area fraction suggests that the contribution to the C- and N-flows of these macroscopically identifiable patches of recent organic inputs is relatively small, or even negligible, compared to the overall C- and N-flow in the soil. This conclusion is based only on the sampling period in spring, half a year after addition of these organic inputs to the soil, but may be valid in other periods of the year too.

A significant spatial correlation between roots and cracks was found in the plough layer, although root density only doubled for zones close to the cracks. Spatial correlation between recent organic inputs and cracks (mainly tillage induced) was indicated, although for a statistical test larger sample areas would be needed, due to the low densities. By combining these effects, a certain degree of synlocation of roots and sites of increased N-mineralization is indi-

cated, but its effect on plant nutrition and on N-use efficiency is probably small.

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Root–soil contact of field-grown winter wheat^α

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ABSTRACT

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Roots following existing macropores and voids normally have only partial root–soil contact. Roots penetrating the soil matrix (creating new macropores) initially have complete root–soil contact. Partial root–soil contact may decrease the roots effectivity in taking up water and nutrients. If all roots have complete root–soil contact, however, aeration may be problematic unless roots have a high air-filled porosity. For field-grown winter wheat, root–soil contact was quantified from horizontally oriented thin sections at three depths, 15, 25 and 45 cm, respectively. One day prior to sampling, surface-connected pores were stained by infiltrating a methylene blue solution. Roots were observed microscopically using polarized light, and their diameter, roundness (indicating orientation) and degree of soil contact were measured with a Quantimet 970 image analyzer.

No relation was found between root–soil contact and root diameter or roundness. At 45 cm depth root–soil contact was less. For two fields, differing in soil organic matter content and current crop management, a different frequency distribution of root–soil contact was found in the plough layer. The percentage of roots with 100% root–soil contact was 65 and 37, that with 0% root–soil contact 5 and 14, respectively. For roots with partial root–soil contact the average degree of contact was about 60% in both cases. Average root–soil contact for the plough layer of the two fields was 84 and 66%, respectively. Roots without direct contact with the soil were growing mostly in surface-connected (blue stained) macropores. There was no difference in blue staining of the macropores with roots with 1–49% root–soil contact and those of the whole sample. Roots with 50–99% root soil contact occurred mostly in relatively small, non-stained pores.

INTRODUCTION

Roots are interacting with soil structure in two ways: they may depend on pre-existing macropores and cracks (voids) to grow, and they may create new

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macropores by penetrating the soil matrix. After decay of the penetrating roots macroporosity can thus be increased. If roots penetrate the soil matrix they will initially have complete (100%) root-soil contact. If they follow pre-existing macropores or voids they will have no or partial contact with the soil, except when they completely fill a pre-existing macropore. Root-soil contact may change during the life of a root. Temporary root shrinkage during plant water stress or decay of epidermis and cortex leads to a decrease of root-soil contact. Secondary thickening of roots may increase root-soil contact.

The degree of root-soil contact is important for both aeration and uptake activities of the root. The chance that roots will obtain sufficient oxygen to survive periods of reduced soil aeration after heavy rainfall decreases with a higher degree of root-soil contact (De Willigen and Van Noordwijk, 1984, 1989). The ability to obtain water and nutrients from the soil matrix, however, increases with increasing root-soil contact (De Willigen and Van Noordwijk, 1987; Veen et al., 1992).

Root-soil contact may change with changing soil water status. Huck et al. (1970) described diurnal shrinkage-swelling cycles of roots under drought stress. Root shrinkage may lead to air gaps around the root and to increased resistance to water uptake (Faiz and Weatherley, 1982; Herkelrath et al., 1977). In these descriptions it is normally assumed that root-soil contact was complete in a wet soil and is reduced by root shrinkage. Actual measurements of the root-soil geometry are required to test these ideas. Thin-section preparation methods as described by Altemüller and Haag (1983) and Altemüller and Vorbach (1987) allow observation of root-soil geometry, including the role of root hairs. Samples are dehydrated by gradual acetone replacement, which is a time-consuming process.

Root-soil contact can also be quantified from thin-sections of the soil made by freeze-drying samples before impregnation of the soil with resin. Previous tests of this method on a pot experiment with maize at three soil porosities showed that roots do not shrink (at least not beyond the normal diurnal range) and that all root cross-sections are recognized (Van Noordwijk et al., 1992). In this pot experiment the average degree of root-soil contact was found to increase from 60 to 87% when soil porosity decreased from 60 to 44% (Kooistra et al., 1992). A study of root-soil contact for field-grown winter wheat was made as part of the Dutch Programme on Soil Ecology of Arable Farming Systems (Kooistra et al., 1989).

METHODS

Observations were made on June 7 1990 on two winter wheat fields at the Lovinkhoeve Experimental Farm in the Noordoostpolder (Marknesse, The Netherlands), with potato as the preceding crop. The soil is a calcareous silt loam with pH-KCL 7.5 and was reclaimed in 1942. At about 45 cm depth

there is a sandy layer of several cm thickness, with many shells, which restricts root development. Below that zone root length density normally increases again. A series of samples of 80 mm $\times$ 150 mm $\times$ 20 mm was taken from a vertical profile wall and horizontally oriented samples were taken at 15, 25 and 45 cm depth (the latter in the middle of the sandy layer), from a single soil pit in two fields: (1) field 16A with a relatively high soil organic matter content (2.7% in the top 20 cm) due to previous management, and currently under "integrated" management, i.e. reduced N-fertilization, reduced pesticide use and shallower soil tillage (12–15 cm cultivator in stead of 20–25 cm ploughing), (2) field 12B with a relatively low soil organic matter content (2.1% in the top 20 cm) due to previous management, and currently under "conventional" management.

Further data on the fields and the experiment are given by Kooistra et al. (1989). One day before the samples were taken, a methylene blue dye solution was infiltrated in the soil to stain surface-connected macropores and voids.

Thin sections, of 25 μ m thickness, were prepared after freeze-drying the samples as described by Van Noordwijk et al. (1992). Vertically oriented samples were used to measure macroporosity ($> 30 \mu$ m diameter). The horizontally oriented samples were scanned under a dissecting microscope, using crossed polarization filters below and above the sample to increase contrast between cell wall material and macropores. All roots were mapped on photographic enlargements (using the thin section as negative), and the root intensity (number of root intersections per cm^2) was measured. Quantitative analysis of roots and pore geometry was done with an image analyzer (Quantimet 970, Leica) at a magnification of $\times 213$ (length of one pixel 1.36 μ m), on 640 fields of view (0.94 mm^2 each) in the thin section. The primary root parameters measured were: number (N), area of cross-section (A), perimeter (P), largest (L) and smallest (S) Feret (caliper) diameter and percentage of root perimeter having direct root-soil contact. From the primary parameters a shape factor [$P^2/(4\pi A)$] was derived indicating the "roundness" of the roots. For a transverse section of a cylindrical root roundness is close to 1.0; for an elliptical shape of a root intersecting the plane of observation at a smaller angle, higher values (at least up to 5.0) are found. In a population of circles and ellipses the roundness factor thus indicates the orientation of roots. Estimates of the angle of interception from $\arcsin(S/L)$ may, however, contain a serious bias (Van Noordwijk et al., 1992). Within the population of roots on a single thin-section, a regression analysis was performed to test whether or not root-soil contact is related to root diameter or roundness. Comparisons of proportions of roots in four root-soil contact classes (0, 1–49, 50–99, 100%) between thin sections were based on a chi-square test.

A series of auger samples (7 cm diameter; 12 per field) was taken on the same day as the soil samples for the thin sections. The auger samples were

washed over a fine sieve (0.3 mm mesh), root length was determined with the line intersect method (Tennant, 1975) and diameter was measured on 20 randomly chosen roots per sample under a dissecting microscope.

RESULTS

Root length density and root diameter in the auger samples and root intensity on the thin sections are given in Table 1. The average root diameter on the thin sections was 0.178 mm; the average for the equivalent auger samples, weighted for root length per sample, was 0.203 mm. As the frequency distribution of root diameters on the thin section was skewed, a logarithmic transformation was used and the few roots of more than 2 mm diameter were discarded. Expressed this way, the average root diameter on the thin sections increased significantly with depth, from 0.161 to 0.208 and 0.242 mm for 15, 25 and 45 cm depth, respectively. No difference in root diameter between the two fields was found.

The average roundness of roots was 1.75. No effects of depth or field on roundness, a rough indication of root orientation, were found. Roundness and root diameter were not correlated either.

Figure 1 presents two of the thin sections showing the two main positions of roots in soil: growing in macropores along aggregate surfaces (A) and penetrating the soil matrix (B). The first group has a partial, the second a complete root-soil contact. Figure 2 shows details of root-soil contact, varying from 0 to 100%.

Figure 3 shows the frequency distribution of root-soil contact observed in

TABLE 1

Root diameter (Diam) and root length density (L_{rv}) observed in auger samples and root intensity (N) observed on horizontally oriented thin section samples for two fields, 12B and 16A

Depth, cm	Diam (mm)		L_{rv} (cm/cm ³)		N (cm ⁻²)	
	12B	16A	12B	16A	12B	16A
0- 5	0.203	0.223	13.2	18.4		
5- 10	0.199	0.197	7.87	10.1		
10- 20	0.193	0.188	3.85	4.98	4.90	4.25
20- 30	0.206	0.227	2.94	3.22	2.85	1.86
30- 40	0.233	0.241	1.49	1.80		
40- 50	0.220	0.239	1.27	1.06	0.81	0.28
50- 60	0.213	0.221	1.44	1.37		
60- 70	0.191	0.217	1.20	1.74		
70- 80	0.187	0.219	0.84	1.47		
80- 90	0.196	0.227	0.28	0.95		
90-100	0.199	0.220	0.12	0.29		

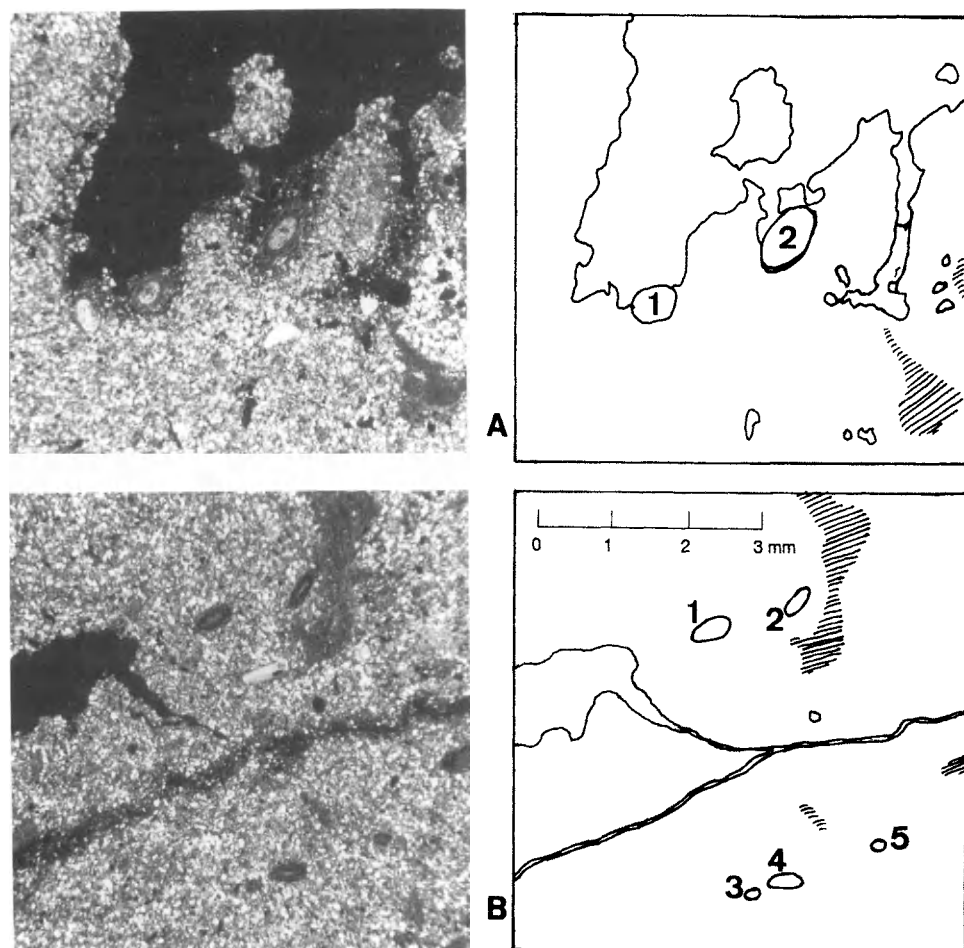


Fig. 1. Overview of two main positions of roots in soil. (A) Two roots growing in a void, along an aggregate wall, with partial root-soil contact. (B) Five roots penetrating the soil matrix, with 100% root-soil contact. Hatched areas on the sketches indicate organic matter accumulations; black areas on the photographs are voids (macropores and cracks).

six thin-section samples. Linear regression analysis showed that no relation existed between root-soil contact and root diameter (only 7% of variation accounted for) or roundness (no variation accounted for). A chi-square test of homogeneity of the frequency distributions indicated ($0.05 < p < 0.10$) a difference between the three depth layers, when results for the two fields were pooled. For a combination of the two samples in the plough layer, a chi-square test indicated a significant ($p < 0.05$) difference between the two fields in frequency distribution of root-soil contact. In field 16A fewer roots with complete root-soil contact (forming their own channel) were found, and for the

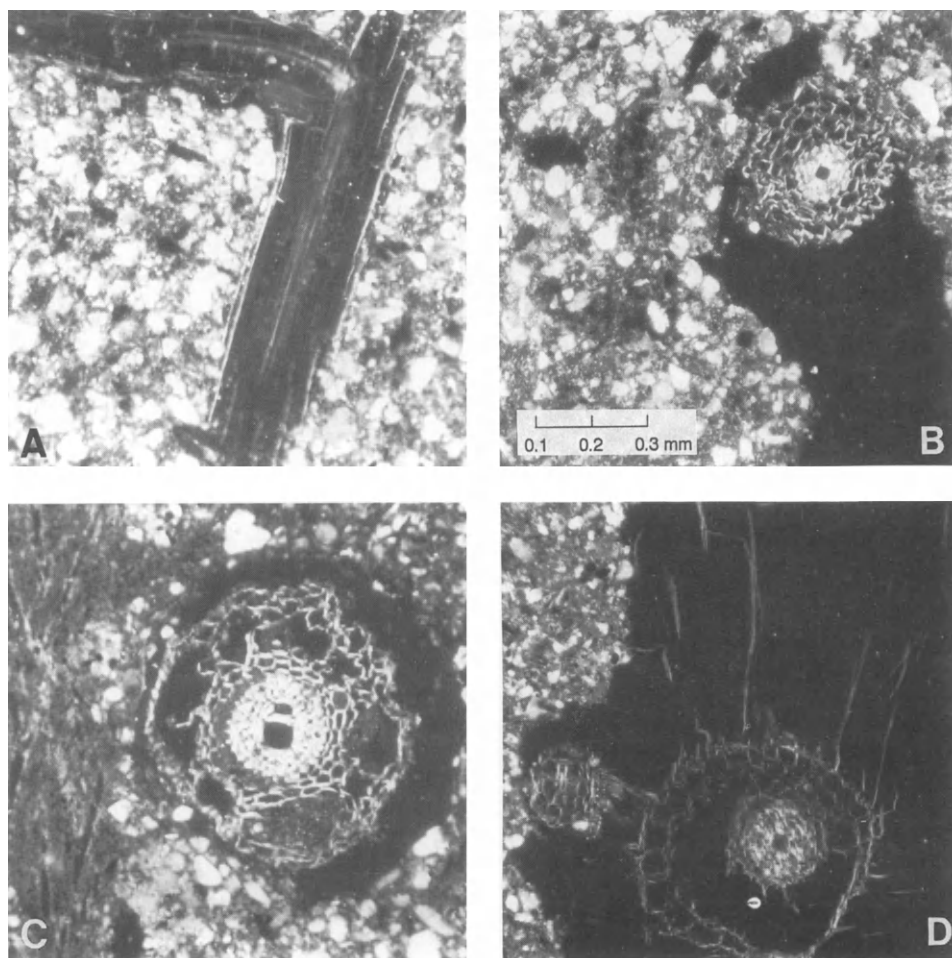


Fig. 2. Details of root-soil contact of wheat as observed on thin sections. (A) Longitudinal section of branching root with 100% root-soil contact. (B) Transverse section of root growing in a void with direct soil contact on two sides, root penetrating the soil matrix. (C) Transverse section of root with partial contact; note cortical aerenchyma. (D) Transverse section of root without direct soil contact, but with root hairs extending towards the soil matrix and a branch root with partial contact.

roots growing in pre-existing macropores a larger part had no direct contact with the soil. In the latter group root hairs may still establish partial contact with the soil, but only on a fraction of the roots without soil contact root hairs were seen. Table 2 summarizes root-soil contact data for the plough layer at the two fields. Average root-soil contact differed significantly between the two fields.

Part of the macropores in which roots were found were stained with meth-

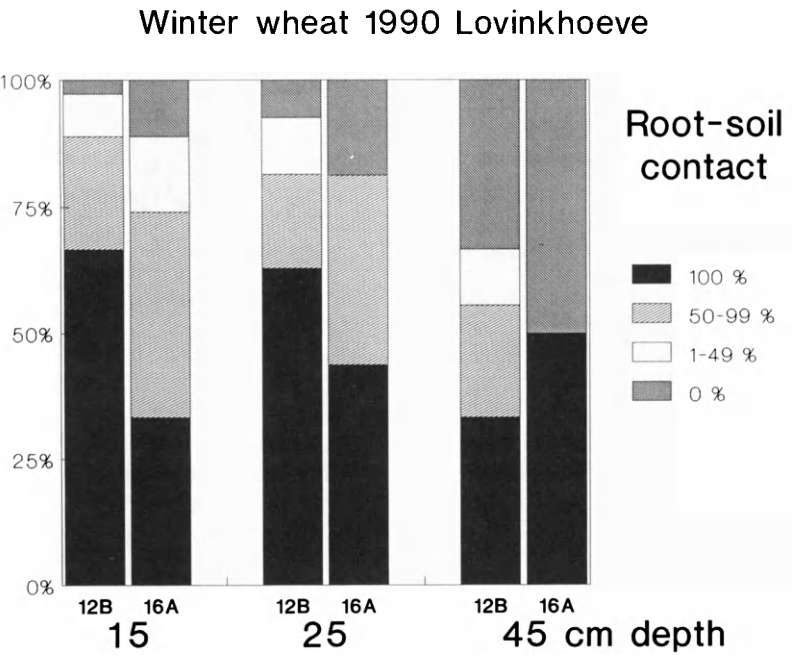


Fig. 3. Frequency distribution of root-soil contact of wheat in horizontal thin-section samples taken at three depths in two fields of the Lovinkhoeve experiment. Number of roots observed in the 640 fields of view (0.94 mm² each) on six thin sections: 36, 27, 27, 16, 9 and 4, respectively.

TABLE 2

Root-soil contact of wheat for horizontal thin sections at 15 and 25 cm depth for two fields, 12B and 16A

Root-soil contact class (%)	Percentage of roots		Average contact	
	12B N=63	16A N=43	12B	16A
0	4.8	14.0	0	0
1-99	30.2	48.8	61.7	58.6
100	65.1	37.2	100	100
Total	100	100	83.7	65.8

ylene blue and are thus considered to be surface-connective. Table 3 shows the macroporosity (diameter > 30 μ m) for vertically oriented thin-section samples, taken close to the horizontally oriented ones used for evaluating root-soil contact. The macroporosity on the thin sections is relatively high, especially on field 16A, compared to previous measurements (Kooistra et al., 1989). About 30% of the macropores in the upper 20 cm of the soil is blue

TABLE 3

Macroporosity ($> 30 \mu\text{m}$ diameter) in thin-section samples for three depth zones of vertically oriented thin-section samples of field 12B and 16A

Depth (cm)	Macroporosity (% v/v)					
	Total		Blue stained		Percentage blue	
	12B	16A	12B	16A	12B	16A
01–09	6.26	19.35	2.04	6.61	33	34
11–19	14.63	23.15	6.81	5.06	46	22
21–29	4.65	6.80	0.79	0.63	17	9

stained, similar to results for 1985 (Kooistra et al., 1989). When the macropores are classified by equivalent diameter, the percentage blue-stained pores tends to increase with diameter. When macroporosity is classified by shape, the percentage blue-stained is about 20% for round pores, about 25% for vughs and about 40% for cracks in the upper 20 cm, averaged over the two fields.

Of the roots growing without direct contact with the soil 56% were growing in blue-stained macropores; even if they occur mainly in relatively large cracks, they appear to prefer blue-stained and thus surface-connective cracks. Of the roots with 1–49% and 50–99% contact 20 and 7%, respectively, were growing in blue-stained macropores. Roots with 100% soil contact were not stained.

DISCUSSION

The average root diameter on the thin sections was 10% less than the diameter of roots washed from auger samples. This might be caused by root shrinkage during sample preparation, or by losses of fine rootlets when washing or by a different criterion for separating live and dead roots. Root shrinkage is probably not a major cause as we observed many roots with 100% root-soil contact. When the thin-section method was tested on a maize pot experiment, with soil without root remains, the root intensity observed (N , cm^{-2}) agreed with the root length density (L_{rv} , cm/cm^3) from washed replicate samples, following the theoretical relation $L_{rv} = 2N$ (Van Noordwijk et al., 1992). For the current samples we found $L_{rv} = 0.97N$ on field 12B and $L_{rv} = 1.3N$ on field 16A, averaged over 15 and 25 cm depth. This could be due to the fact that the thin section samples had a higher than average root density (the data fall within the range of root densities on individual auger samples) or to the fact that part of the roots observed on the thin section would be discarded as dead roots in the washed samples. In discussing the data the possibility of the latter explanation should be kept in mind.

Roughly two thirds of the roots formed their own root channels on the thin

sections of field 12B, as derived from the frequency of 100% root-soil contact, and only one third on 16A. The considerably higher macroporosity of the plough-layer samples of field 16A, with its higher soil organic matter content, suggest that more roots could follow pre-existing pores on that field, while roots on field 12B had to penetrate the soil matrix. Roots occupied only a small fraction (1–5%) of the available, blue-stained macropore space, however, even on field 12B. The small size of the thin section samples, taken from one soil pit in each field, makes it impossible to extrapolate from the samples to the field scale. Because cropping history and current management are confounded in the comparison of the two fields (Kooistra et al., 1989), no conclusions on the influence of either of them can be made. The differences found in root-soil contact are primarily related to a difference in macroporosity, not to specific causes of this difference in macroporosity.

The average values for root-soil contact in this study are within the range found earlier for maize (Kooistra et al., 1992). Root hairs which may make up for incomplete root-soil contact were seen only rarely, although some examples show that they survive sample preparation without apparent damage. As noted in a pot experiment (Kooistra et al., 1992), roots growing in existing macropores and cracks have more contact with the soil than would be expected for a random positioning in the macropore space. Roots can apparently find and follow the walls of the macropores and cracks.

Roots without direct contact with the soil were growing mostly in surface-connected (blue-stained) macropores. Roots with 1–49% root-soil contact occurred in macropores with an average fraction of blue staining, while roots with 50–99% root-soil contact occurred mostly in relatively small, non-stained pores. Roots penetrating the soil matrix (100% root-soil contact) probably depend on internal aeration through cortical air spaces. A certain part of each root axis should have air contact to serve as “breathing sections”. The observation that roots with complete air contact grow mostly in surface-connected pores agrees remarkably well with this interpretation.

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The influence of Enchytraeidae (Oligochaeta) on the soil porosity of small microcosms

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ABSTRACT

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Tunneling and burrowing activities of soil organisms affect structure and porosity of soils, but the role of enchytraeids as a soil burrowing organism is unclear. This study was initiated to evaluate the effect of enchytraeids on soil porosity in two soils with different texture and similar, low organic matter contents.

In small microcosms, the burrowing activities of *Enchytraeus minutus* were followed during a 51 day period. The microcosms consisted of two glass plates with a bottom of plaster of Paris and sides of Plexiglas. Sieved (< 1 mm), enchytraeid-free soil was added between the glass plates and two litter placement treatments were simulated by the addition of rye to the surface or by incorporating it into the soil. The soil in the microcosms was wetted from the bottom through the capillary action of the plaster and soil. Twenty enchytraeids were added to the microcosms and every 17 days the distribution of pores was determined under a stereomicroscope by a point counting technique and with image analysis.

At least 65% of the enchytraeids survived in the microcosms, and in the surface litter treatments, the population expanded. Enchytraeids increased porosity in the microcosms at 17 days. At later times the porosity of the microcosms decreased to levels below the starting values. This decrease in porosity was attributed to consolidation of the soils as a result of overburden pressure and destabilization of soil aggregates caused by the egestion of the soil by enchytraeids. Porosity estimates by image analysis were much lower than by point counting, but a strong correlation was found between the two.

INTRODUCTION

Porosity is one of the most important properties of soils. A thorough knowledge of porosity is essential to solve problems concerning soil tillage, land

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amelioration, water management, rooting, etc. (Jongerijs et al., 1972). Porosity investigations are presently very important for the understanding of groundwater contamination by leaching of fertilizers and pesticides.

Tunneling and burrowing activities of soil organisms have a significant influence on the structure and porosity of a soil. Górný (1984) stated that enchytraeids were unable to burrow channels; for vertical migration they were dependent upon the smaller pores present in the soil. Alternatively, Jegen (1920) and Rusek (1985) concluded that enchytraeids can make microtunnels in the soil matrix. Standen (1984) suggested that large enchytraeid species, like *Fredericia galba*, formed burrows and transported or deposited material as faeces to a depth of 3.5–4.0 cm. Species of other genera did not form visible burrows but material was transported 0.5 cm in some cases. Didden (1990) concluded that *Enchytraeus buchholzi* Vejdovsky 1879 influenced the pore size distribution or the pore continuity of soils. A number of researchers (e.g. Zachariae, 1964; Babel, 1968; Pawluk, 1987) have pointed to the occasionally large numbers of enchytraeid faecal pellets in mor and moder humus soils. Others (e.g. O'Connor, 1967; Babel, 1968; Ponge, 1984) have mentioned the transport of mineral particles by enchytraeids, either by ingestion or attached to the body surface. From these results it seems reasonable to assume that enchytraeids influence soil structure in a significant manner.

In 1964 Van der Plas and Slager reported a method to study the distribution of biopores in soils. Their observations on structural properties of a soil profile were carried out with a stereomicroscope and with slices of a soil profile.

Image analysis of soil thin sections is presently very popular. It provides an estimate of the total porosity and a quantitative morphological characterization of the soil porosity. However, image analysis is subject to errors due to the dark colors of features other than voids (e.g., fungi colonies), to exposure time in photography and to subjectivity in setting the threshold values (Delgado and Dorronsoro, 1983).

Christensen (1956) built breeding chambers out of 2 observation slides and cardboard, which was cut into a U-shape. Christensen used the breeding chambers to study the reproductive biology of enchytraeids. The design of the microcosms used in this experiment is a combination of the methods of Christensen and Van der Plas and Slager.

The objectives of this study were (1) to determine the suitability of the developed microcosms for experiments with Enchytraeidae; (2) to determine the influence of Enchytraeidae on the soil porosity of 2 types of soils with low organic matter content and different texture; (3) to determine the influence of Enchytraeidae on the soil porosity of 2 litter placement treatments, buried versus surface litter, which simulate conventional tillage and no-tillage agroecosystems; and (4) to compare porosity estimates by the point count method and with an image analysis system.

MATERIALS AND METHODS

Pre-treatment of the soil

Two soil surface horizons, a sandy clay loam (SCL) and a loamy sand (LS) (from clayey, mixed, thermic Typic Kanhapludults), both low in organic matter content were selected for this experiment. Soil characteristics are listed in Table 1. The air-dried soils were passed through a 1 mm sieve, and the < 1 mm separate was heated in a water bath to 50°C for 4 hours to exterminate any enchytraeid cocoons present (Christensen, 1956). This heating method also affects some microbial populations, therefore the soil was reinoculated with soil extract (a mixture of 500 g soil in 1 l water, which was filtered through No. 1 Whatman filter paper (11 µm particle retention)). After a second air-drying the soil was added to the microcosms.

The microcosms

The microcosms consisted of 2 glass sheets (18 cm × 27.5 cm) which were held apart by a 0.3 cm thick layer of Plaster of Paris at the bottom [ratio of plaster (g) to water (ml) is 1.9:1] and Plexiglas at the sides (Fig. 1).

Two soil management treatments were simulated by the addition of 0.8 g of rye (*Secale cereale* L.) to the surface (SL, surface litter) or by incorporating it into the soil (BL, buried litter) before the soil was added to the microcosms. Each microcosm was filled from the top with air-dried soil aggregates smaller than 1 mm. For the SCL treatments 65 g of soil were used and for the LS, 90 g. The height of the soil column in the microcosms varied between 13 and 15 cm. The litter layer in the SL treatments was approximately 1 cm thick. Bulk densities of the microcosms were 1.2 and 1.25 g/cm³ for the sandy clay

TABLE 1

Characteristics of the 2 soil types used in the microcosms

	SCL	LS
Total C (%)	1.6	0.7
Total N (%)	0.15	0.05
C:N	10.6	14.0
Soil texture		
Silt (g/kg)	136–175	95–138
Clay (g/kg)	130–136	55–106
Amount used in microcosm (g)	65.0	90.0
Amount of rye litter used in microcosm (g)	0.8	0.8

SCL = sandy clay loam; LS = loamy sand.

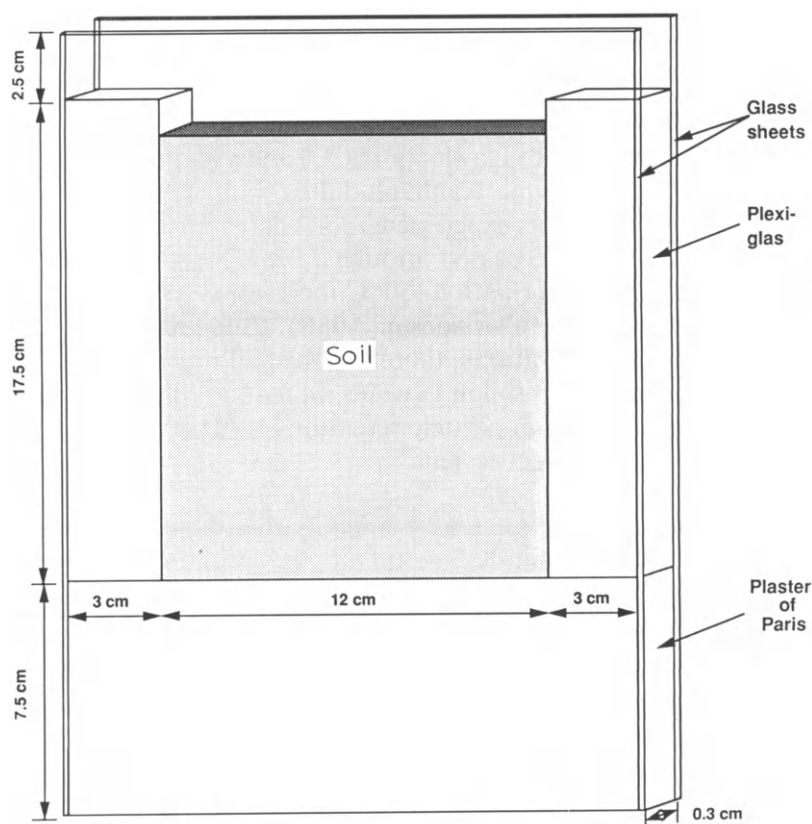


Fig. 1. Diagram of a microcosm used in the experiment.

loam BL and SL respectively; the loamy sand microcosms had bulk densities of 1.73 and 1.76 respectively for the BL and SL treatments. Every soil-tillage-enchytraeid combination consisted of 3 replicates. The microcosms were placed in a pan with water (0.1 to 0.5 cm deep) and the soil in the microcosms wetted from below through the capillary action of the plaster and soil. The porosity of each microcosm was measured before the experiment (T_0) and every 17 days up to 51 days (T_1 , T_2 and T_3) with the point count method. At T_0 and T_3 photographs for image analysis were taken as well.

After time zero (T_0) porosity estimations, twenty enchytraeids (*Enchytraeus minutus* Nielsen and Christensen 1961) were added at the top of the soil or litter layer to half of the replicates for each soil and tillage treatment. The organisms had an average length of 6 mm and a width of 0.3 mm. This species forms burrows in SCL and LS soil cultures (personal observation). The microcosms were stored in a dark room with the temperature fluctuating between 20 and 24°C, which simulates the temperatures in late spring or early

fall in Georgia. After T_3 the microcosms were taken apart to determine the moisture and enchytraeid distribution.

Porosity measurements

The porosity of each microcosm was measured by Chayes' (1956) point count method by identifying the status of the point as being a pore or a solid using a stereomicroscope and a magnification of $10\times$. No size limit of the pore was imposed. A grid overlaying the microcosm was used and in the horizontal direction a point was recorded every 0.5 cm; vertically, a point was recorded every 1 cm. To avoid any edge effects, counting started 1 cm below the surface and 1 cm away from the Plexiglas sides. For the upper 12 cm of each microcosm, 228 points were counted which were divided into 3 depth increments 1–4 cm, 5–8 and 9–12 cm. Porosity was estimated by the number of pores found in the number of points counted.

To perform a porosity estimation with an image analysis system, black and white photographs (Kodak TMX 100 film) were taken through a stereomicroscope at a magnification of $10\times$. A series of photographs, each encompassing 1 cm vertically, was taken from the surface to the bottom of the microcosm. Two columns of photographs were taken from each microcosm, and the negatives were analyzed for total porosity with an Olympus Cue-2 digital imaging system. An area of 52 mm^2 was analyzed on each negative and small objects (smaller than 2×2 pixels, 0.0012 mm^2) were removed from the image. Porosity estimates per cm for the two columns and for the 1–4, 5–8 and 9–12 cm depth increments were combined for subsequent analysis.

To determine the influence of enchytraeids on the porosity of the microcosms a proportional change in porosity (P_i/P_0) was calculated (i =time T_1 , T_2 or T_3 and $0=T_0$). These ratios were analyzed using the ANOVAR (analysis of variance repeated measures) procedure in SAS (SAS®, 1988). Because the ratios were normally distributed, no transformation was applied to the data. To compare the porosity estimates of the point count method and the image analyzer, a correlation analysis was performed for the T_0 and T_3 estimates.

RESULTS

Suitability of the microcosms

The suitability of the microcosms for enchytraeids can be determined by evaluating the recovery data of the different microcosms (Table 2). A larger number of enchytraeids was found in the SL microcosms than was added at the start of the experiment. Most of these enchytraeids were juveniles and were found in the litter layer. Apparently, conditions in the SL microcosms

TABLE 2

Mean number of enchytraeids recovered in the different microcosms

	BL			SL		
	SCL	LS	BL _{avg}	SCL	LS	SL _{avg}
Litter	–	–	–	83.3 (46.0)	34.7 (10.5)	59.0 b (23.7)
0– 4 cm	2.0 (1.2)	5.0 (4.5)	3.5 a* (2.2)	9.7 (2.4)	9.0 (2.6)	9.3 a (1.6)
5– 8 cm	1.7 (0.9)	2.0 (1.0)	1.8 a (0.6)	4.0 (3.1)	5.7 (1.5)	4.8 a (1.6)
9–12 cm	5.7 (0.9)	3.0 (1.5)	4.3 a (1.0)	4.7 (3.7)	12.7 (3.5)	8.7 a (2.9)
12–. cm**	6.3 (0.9)	3.0 (1.2)	4.7 a (1.0)	5.0 (2.6)	33.3 (10.9)	19.2 b (8.1)
Total	15.7 (1.5)	13.0 (57.1)		106.7 (57.1)	95.3 (14.4)	

$n=3$, standard errors in parentheses. SCL=sandy clay loam; LS=loamy sand; BL=buried litter; SL=surface litter. The effect of litter placement on the total number of enchytraeids was significant ($p<0.05$).

*For the effects of depth increment, means in a given column followed by a common letter are not significantly different at $p<0.05$.

**Contains the amount of soil from 12 cm below the surface to the beginning of the Plaster of Paris (varied between 13 and 15 cm depending on the soil and litter placement treatment).

had been favorable for the enchytraeid population to expand. In the BL treatment microcosms, 65–80% of the enchytraeids were recovered. In all treatments some enchytraeids might have been lost to subsampling for moisture determination (2 g of soil) and KCl extractions (2 g of soil).

Within a soil texture and litter treatment, all depth increments had a similar moisture content. The moisture content of the LS soil (16% w/w) was half the moisture content of the SCL soil (36% w/w) (significant at $p<0.0001$), which is due to the lower clay and silt content. The buried litter treatment (BL) had a significantly ($p<0.0001$) higher moisture content (28% w/w) than the surface litter treatment (SL) (24% w/w). This may be because of additional water retention by the incorporated organic matter. The presence or absence of enchytraeids did not affect the moisture profile.

The distribution of the enchytraeids and the moisture profile were evaluated at the end of the experiment only. The soil may have been drier or wetter depending on the amount of standing water in the pan (0.1–0.5 cm).

Point count results

Other studies have found a difference in distribution in enchytraeids in no-tillage (NT) and conventional tillage (CT) agro-ecosystems (Didden, 1988;

Van Vliet, unpubl. data). In NT systems enchytraeids are mostly present in the top 5 cm of the soil, while in CT systems enchytraeids are more evenly distributed over the soil profile. Therefore, changes in the porosity in the SL microcosms were expected in the 1–4 cm depth increment, while changes in porosity in the BL microcosms were expected to be more evenly distributed.

Looking at the SCL-BL and -SL point count method results (Fig. 2), we conclude that in the treatments without enchytraeids consolidation of the soil occurred due to slaking and particle rearrangement as the soil remained in a moist condition. Consolidation was less in the SCL-BL than in the SCL-SL treatment apparently because of the stabilizing effect of incorporated organic matter. Other studies have shown that the presence of organic matter in the soil stabilizes soil structure (reviewed in Stevenson, 1986 and in Tate, 1987).

In the SCL-BL treatment (Fig. 2A–C), enchytraeids caused an increase in porosity at 17 days at all depths. After 17 days, the porosity of all depth increments decreased to levels below the starting values. In the SCL-SL treatment (Fig. 2D–F), enchytraeids influenced the porosity significantly at the 1–4 cm depth (ANOVAR, significant enchytraeid effect, $p=0.08$) and appear to have slowed consolidation at the two lower depths since porosity estimates were higher than the porosities in the no-enchytraeid microcosms until 34 days. At 51 days, the porosity at all depths decreased in the SL treatment to levels similar to or less than the microcosms with no enchytraeids.

Figure 3 shows the results of the point count method for the LS treatments. At all depth increments of the LS-BL no-enchytraeid treatment the porosity increased through day 34. In the presence of enchytraeids, the porosity of the microcosms increased at all depths at day 17. Only at the 1–4 cm depth increment was the increase in porosity significantly larger ($p<0.10$) than in the microcosms without enchytraeids. At later times, enchytraeids diminished the effect of the increasing porosity measured in the no-enchytraeid treatment. In the LS-SL treatment enchytraeids increased the porosity in the 1–4 cm depth increment at day 17. Consolidation of the soil at the 9–12 cm depth increment was more severe in the microcosms without enchytraeids than in the microcosms with enchytraeids (ANOVAR, significant enchytraeid effect at $p<0.05$). A large number of enchytraeids was found in the 9–12 and 12– cm depth increments (Table 2); their excrements and exudates might have increased the aggregate stability at the 9–12 cm depth increment.

Enchytraeids affected the porosity mainly in the SCL soil where they initially increased porosity and later stimulated the consolidation of the soil, especially in the SCL-BL treatment. Due to the more rigid particle structure of the LS soil a large edge effect between the glass and the soil was present. Enchytraeids could move between the particles and the glass without altering the soil structure. This edge effect did not occur in the SCL soil. The edge effect in the LS soil complicated the porosity measurements.

In the SL treatment of the SCL soil enchytraeids influenced the porosity

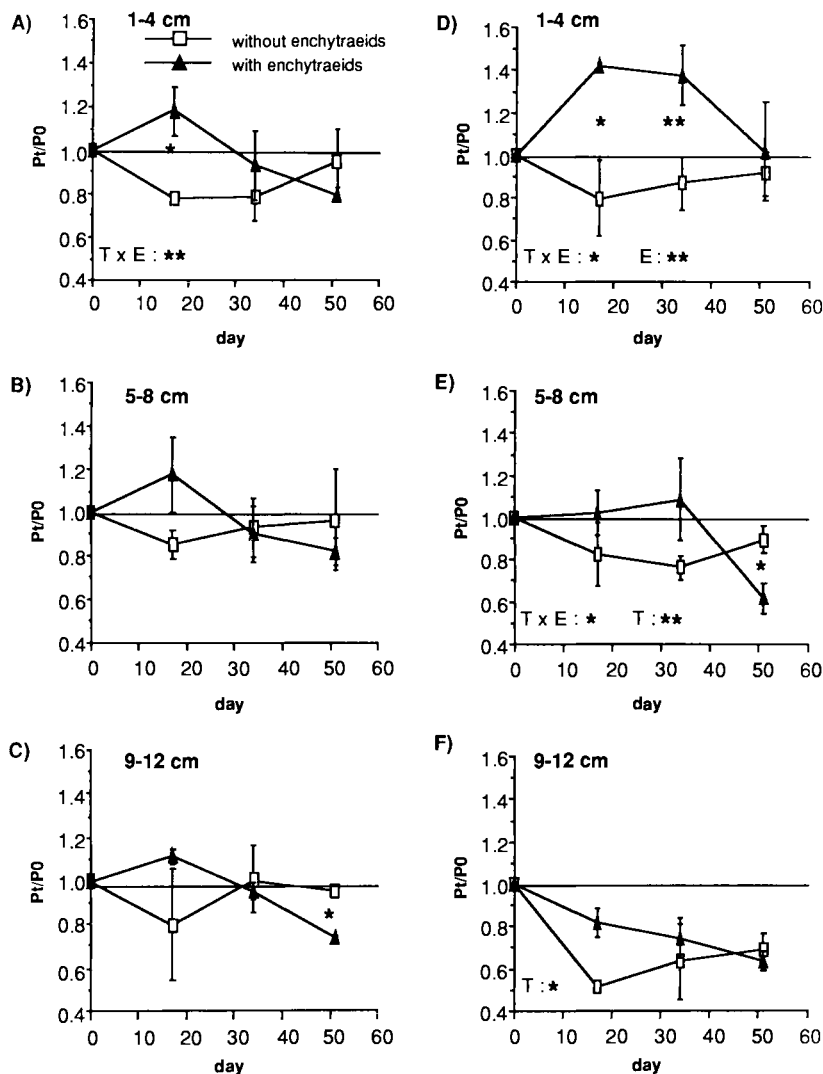


Fig. 2. Proportional change in the porosity (P_i/P_0) over time for the 3 depth increments in the sandy clay loam buried litter (A, B and C) and surface litter microcosms (D, E and F). Significant results from ANOVA are reported in the lower left corners of the graphs. E =enchytraeid effect; T =time effect; $T \times E$ =time $\times$ enchytraeid interaction; *=significant at $p < 0.05$; **=significant at $p < 0.1$. Stars in the graphs denote significant contrasts between the enchytraeid treatments.

mainly at the 1–4 cm depth increment while in the BL treatment lesser effects were found at all depth increments. This difference in the BL and SL treatment might be due to a difference in distribution of the enchytraeids in the BL and the SL. The constantly high moisture content of the SCL soil (36%)

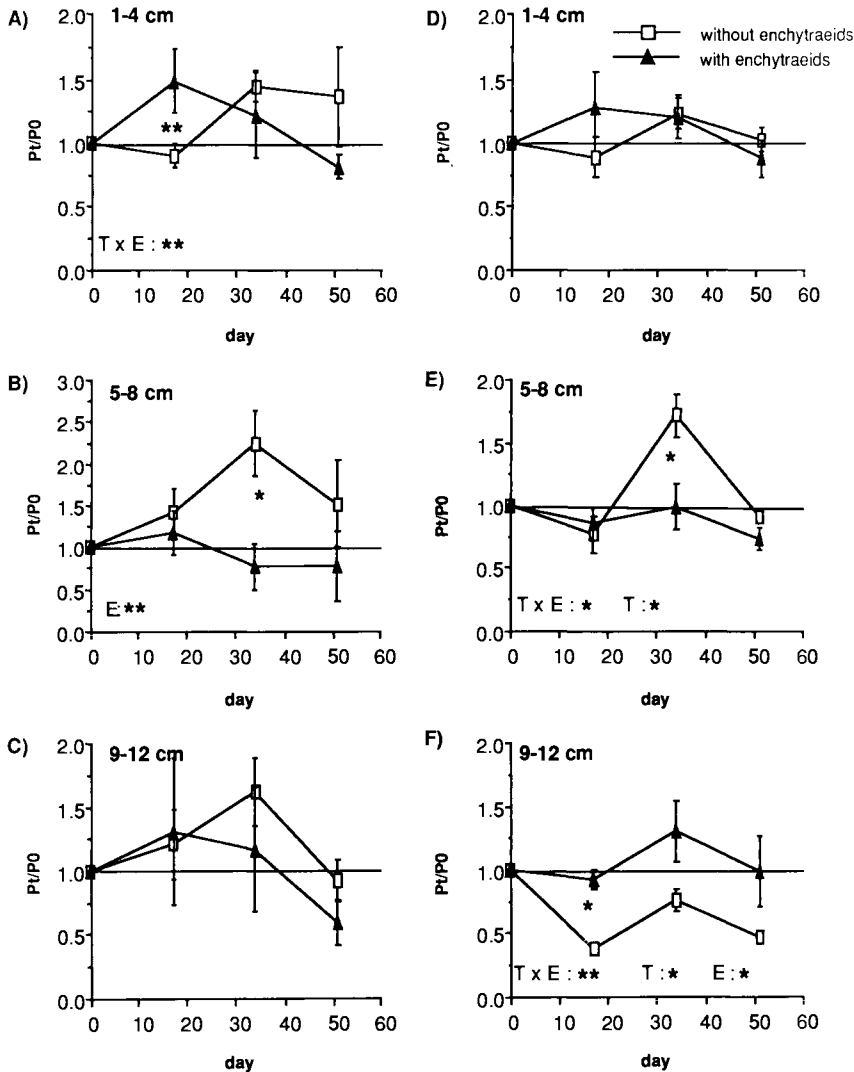


Fig. 3. Proportional change in the porosity (P_t/P_0) over time for the 3 depth increments in the loamy sand buried litter (A, B and C) and surface litter microcosms (D, E and F). Note: the y-axis of (B) is scaled differently. Significant results from ANOVAR are reported in the low left corners of the graphs. E =enchytraeid effect; T =time effect; $T \times E$ =time $\times$ enchytraeid interaction; * = significant at $p < 0.05$; ** = significant at $p < 0.1$. Stars in the graphs denote significant contrasts between the enchytraeid treatments.

and the egesting of the soil by the enchytraeids might have destabilized the aggregates. Enchytraeids might have decreased porosity by filling pores with their excrements. Twenty enchytraeids were added to the microcosms which equals an enchytraeid density of 56,000 per m^2 . In agricultural systems yearly

enchytraeid densities range from 5700 to 30,000 per m² (Ryl, 1977, 1980; Didden, 1991; Lagerlöf et al., 1989; Van Vliet, unpubl. data). Although the density of enchytraeids used in the microcosms seems high compared to the yearly averages, enchytraeids tend to have an aggregated distribution, therefore a high number per area is possible. But the presence of a large enchytraeid population in the microcosms might have led to a second digestion of the soil aggregates which might have reduced the stability even further.

If a drying cycle had been included in the experiment consolidation of the soil and stimulation of consolidation by enchytraeids might have been less severe. The stability of enchytraeid fecal pellets might be affected by ageing and drying similar to earthworm casts (Shipitalo and Protz, 1988, Marinissen and Dexter, 1990)

Comparison of the pore count method and the image analyzer

Table 3 shows the results of the correlation analysis of the porosity estimates with the point counting method and the image analyzer. Although the estimates by the image analyzer were much lower than from the point counting method, the correlations between the 2 methods for all depth increments were very strong ($p < 0.0007$; Pearson coeff. > 0.58).

Due to the exclusion of pores smaller than 0.0012 mm² in the image analysis, the porosity estimates were lower than the porosities determined by the point count method. Also, uneven lighting during photography of the microcosms complicated thresholding and resulted in smaller voids not being de-

TABLE 3

Average porosities (%) determined by the point count method (PC) and the image analyzer (IA) for the different depth increments

Time	Depth	N	PC	IA	Pearson coeff.	p
T ₀	1- 4	30	18.7	8.9	0.62	0.0003
	5- 8	30	20.7	8.6	0.58	0.0007
	9-12	30	22.5	8.5	0.67	0.0001
	1-12	30	20.6	8.7	0.68	0.0001
	All depths	120	20.6	8.7	0.62	0.0001
T ₃	1- 4	30	16.8	6.1	0.80	0.0001
	5- 8	30	18.4	5.7	0.65	0.0001
	9-12	30	18.6	6.1	0.67	0.0001
	1-12	30	18.0	6.0	0.76	0.0001
	All depths	120	18.0	6.0	0.70	0.0001

The Pearson coefficient and the p value are given for each correlation analysis. N is the number of observations per analysis.

tected. McKeague and Fox (1987) compared porosity assessments by some macro- and micro-methods. They concluded that porosity estimates by the point count method and image analysis were comparable.

Another important consideration in the evaluation of these two methods is the equipment needed and the amount of time involved in the analyses. For the point count method only a stereo microscope and sufficient light sources are needed. A lightbox, a camera, a monitor, a computer and an image analyzer software program are needed for analysis on the image analyzer. Only 0.5 h was required to estimate the porosity of a microcosm with the point count method, while the image analysis method (including photographing, film development and analysis) required 1.5 h per microcosm. If a setup with a stereomicroscope is possible, the microcosms could be directly analyzed on the image analyzer, thus decreasing analysis time by 50%. Another advantage of using image analysis is that other characteristics of the pores (e.g. area, perimeter, orientation) are measured.

CONCLUSIONS

The Plexiglas/glass/Plaster of Paris microcosms seems to be suitable for the maintenance and study of enchytraeids. Most of the enchytraeids survived, and in the surface litter treatment, the enchytraeid population increased. The moisture content of the microcosms can be varied by changing the length of the Plaster of Paris and/or the length of the soil column. The microcosm is less appropriate for coarse particulate soils; the larger particle structure of the LS soil caused edge effects between the glass and the soil.

Enchytraeids increased the porosity in the microcosms at 17 days. In the SL treatment, enchytraeids mainly affected the porosity at the upper depth increment (1–4 cm) while in the BL treatment they affected the porosity to greater depths. At later times, the porosity of the microcosms decreased to levels below the starting values. We hypothesize that the enchytraeids have refilled the pores with excrements and have decreased the aggregate stability by passage of the soil through their gut.

This study showed that the point count method was comparable to the image analysis method for estimating soil porosity, as long as relative efficiencies of each are known.

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Estimating orientation and width of channels and cracks at soil polished blocks—a stereological approach

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ABSTRACT

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In most cases soil structure is anisotropic, i.e., its properties depend on the direction in which it is observed. This is true for important soil functions such as rooting potential and transport processes.

The interpretation of micromorphometric results from two-dimensional sections is often biased by anisotropy of the investigated structural elements. Channels and cracks are elongated components of the soil pore system. They may often cause anisotropy of soil structure.

In this paper parameters and stereological procedures are presented for the estimation of 3D orientation and width distributions of channels and cracks when measurements are taken from 2D polished blocks.

Moreover, a method of image analysis is presented by which measuring is done directly on the microscopic image. The results are illustrated by an example.

INTRODUCTION

Within the last two decades, soil research has increasingly focused on soil structure, i.e., the spatial arrangement of voids and solids. Numerous investigations on water transport (e.g. Beven and Germann, 1982) and gas exchange (e.g. Frede, 1986) have demonstrated that soil structure must be regarded as anisotropic space, which means that it exhibits “different properties in different directions of space” (Weibel, 1979). Anisotropy of soil structure often causes difficulties in interpretation of experimental results. In soil physics, most models are designed for isotropic soils, in morphometric investiga-

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tions, the possibilities of interpretation are often limited when anisotropy of the measured elements must be supposed (Ringrose-Voase, 1991).

Anisotropy of transport processes in soil, which is found in measurements of water and air permeability or of gas diffusion at low suctions, is a consequence of morphological anisotropy, which may be observed in polished blocks and thin sections on a scale of 10 to 1000 μm .

Morphologically, several types of void shapes may be distinguished (e.g. Brewer, 1964), among them are channels, which are formed by roots or animals, and cracks, which result from, e.g., shrinkage and swelling. Channels and cracks are elongated structural elements, therefore they may be an important cause of anisotropy.

Information about orientation distribution of channels and cracks, i.e., of direction and degree of anisotropy, gives a better insight into processes influenced by soil structure. This is of interest, for instance, for modelisation of these processes. Moreover, this information is helpful for unbiased estimations of surface density, length density and width of channels and cracks.

It is possible to develop stereological methods for the estimation of 3D orientation distribution of channels and cracks on the basis of 2D sections. To apply these methods, there are some requirements concerning the soil, the way of sampling and preparation, and the measurements themselves. The aim of this paper is to propose stereological procedures to estimate the distribution of orientation and width of channels and cracks from 2D polished blocks, and to discuss the requirements for their application.

SAMPLES

Undisturbed soil samples are taken with Kubiena boxes ($4 \times 6 \times 8$ cm). The Kubiena boxes are inserted laterally into a vertical face of a pit to make sure that the direction of the samples is known. The samples are treated with the acetone method of Fitzpatrick and Gudmundsson (1978) for dehydration without changes in structure. After impregnation with a polyester resin (Vestopal 120), vertical sections are prepared. The section planes are ground and polished and finally etched with 10% fluoric acid. In this way solid and pore appear in good contrast when illuminated with oblique incident light.

Direction of the section plane

The orientation of the section plane must be defined when orientation distribution is to be estimated from planar sections. For channels and cracks in soils, vertical sections are best suited, as results from the following considerations: when the soil surface in the field is horizontal and when the plant stand is homogeneous, no preferred orientation in the horizontal plane can be expected (no preferred orientation with respect to the cardinal points of

the compass). That means the hypothesis may be adopted that there is rotation symmetry with respect to the vertical axis. The same assumption was made by McBratney and Moran (1990).

IMAGE ANALYSIS

Pore types

There are manifold processes of shape formation and of their interrelations and, consequently, there are manifold shapes of pores. But there are a few basic types of pore shapes which can be differentiated in planar sections. According to Brewer (1964) there are four main classes, namely channels, planes, packing voids and vughs. Since then, this classification is frequently used in investigations about morphology of pore systems. In this way information is obtained about soil structure genesis, while, it is also possible to define and measure specific morphometric parameters for the individual shape classes of pores.

The differentiation of channels, cracks and other pores is the first step in the stereological treatment of their orientation and width distribution. In the planar section, the profile of channels is circular to elliptical, the profile of cracks is elongated with more or less parallel boundaries. By automatic image analysis shape factors of individual profiles can be calculated. Pore types are differentiated in this way by several authors (Murphy et al., 1977; Bullock and Murphy, 1980). The most highly developed approach was presented by Ringrose-Voase and Bullock (1984).

In practice, however, there are some problems:

— Shape factors can be calculated only for individual, discrete profiles, as, when the profiles are interconnected, the basic parameters for the calculation, which are area, perimeter and diameter, are not unambiguously defined. Interconnected profiles are frequent and they must be separated “interactively” by the operator.

— The profiles of channels which are cut by the section plane at a very low angle are elongated. Their shape factors are within the range of those of cracks. This may cause a bias when working with vertical sections, in which more or less vertical channels always have very elongated profiles. Most of such profiles cannot be distinguished even by experienced operators from those of cracks in digitized images. But this distinction can be made when working directly at the stereomicroscopic image of the polished block. Then, within the pore, the third dimension can be observed.

These considerations led to the development of a technique of measurement with which direct work at the microscope is possible. Here the assignment of the pores to shape classes must be made by the operator. For this, clear definitions, which may be supplemented by drawings or photographs, are required.

Equipment

In contrast to automatic image analysis, the microscopic image is not transferred by a video camera into a computer but, vice versa, the computer screen is transferred into the microscope. The evaluation takes place directly at the microscope. The principle has been published by Glaser et al. (1983).

The screen of a computer is projected into the path of rays of a microscope by a slightly modified Zeiss drawing device. Thereby, both the computer screen and the microscopic image can be seen in the microscope in one plane (Fig. 1).

Defined geometric shapes created on the computer screen can be adapted by the operator to the objects of interest. In this case the equipment acts like an eye-piece micrometer, whereby direct measurements of lengths and directions can be carried out very quickly.

The microscopic slice is moved on a mechanical stage by computer-controlled motors. Thereby the coordinates within the slice are always known and consequently, the size of the investigated area becomes independent of the size of the microscopic field of view, meaning independence of the magnification applied.

Using a "mouse", all operations of measuring and moving the slice can be

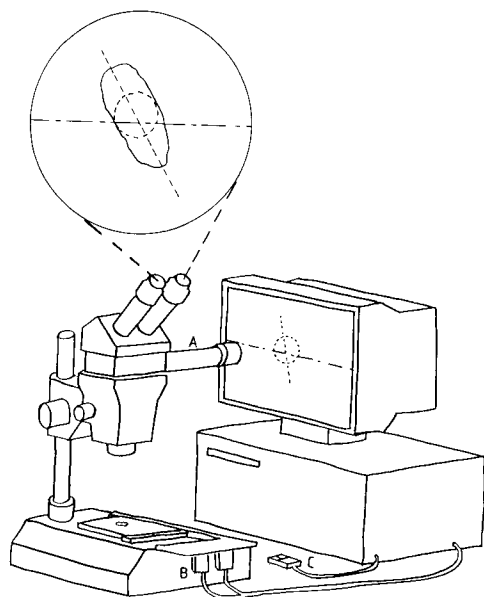


Fig. 1. Equipment for projecting a computer screen into the path of rays of a stereomicroscope by a drawing device (A). The polished block is moved by computer-controlled motors (B). All operations are carried out using a "mouse" (C).

carried out without interrupting microscopic observation. The evaluated data are stored and processed directly in the computer.

Procedure of measurement

All work is done at a stereomicroscope with magnification $40\times$. The polished block is moved along horizontal and vertical testlines with a distance of 2 mm. Points of measurement are all transitions of the testline from solid to void (only voids larger than $30\text{ }\mu\text{m}$ are considered). Thus a systematic choice of points of measurement is achieved. (This is a prerequisite for the application of the stereological methods which are described later on.) At each point of measurement, at first the channels and cracks are discriminated according to the following definitions:

— channels: circular to elliptical profile, at least 75% of the boundary is convex, less than 50% of the profile are infilled. The observable 3D shape is that of a cylinder which corresponds to the profile. Some drawings are shown in Fig. 2.

— cracks: elongated profile, at least five times longer than wide, boundaries approximately parallel. The observable 3D shape is that of a sheet which corresponds to the profile. Some drawings are shown in Fig. 3.

Then the following parameters, which are required for the stereological formula given later on, are measured:

— for channels: smallest diameter (a), largest diameter (b), angle between the largest diameter and the vertical axis (θ') (Fig. 4).

— for cracks: apparent width (τ'), angle between the profile and the vertical axis (θ') (Fig. 5).

With the given equipment the measurements are done as follows:

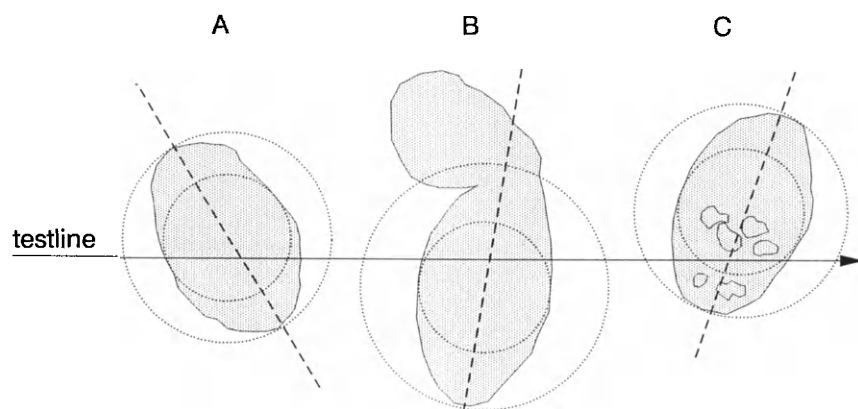


Fig. 2. Examples for measurements at profiles of channels, most frequent (A) and rarer cases (B,C). The circles and chords (dotted lines) are adapted by the operator.

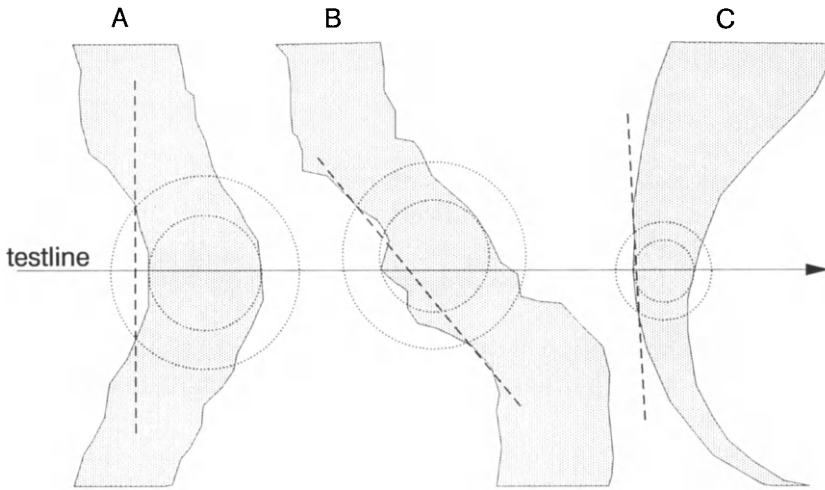


Fig. 3. Examples for measurements at profiles of cracks, most frequent (A) and rarer cases (B, C). The circles and chords (dotted lines) are adapted by the operator.

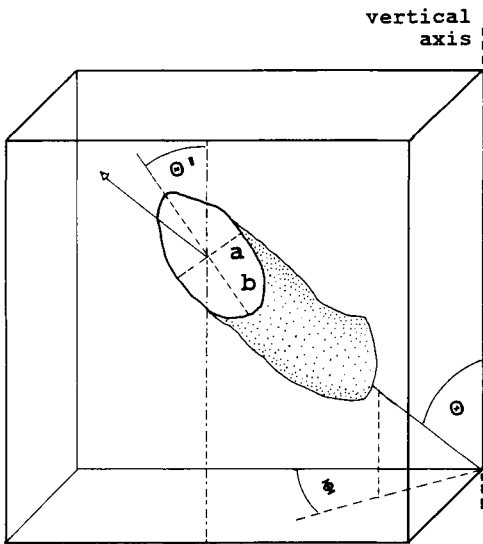


Fig. 4. Geometry of a channel cut by a section plane (front side of the cube). The spatial direction indicated by the arrow is defined by θ and ϕ ; measurements of a (small diameter), b (large diameter) and θ' (apparent angle to the vertical axis) can be taken from the ellipse visible in the section plane.

For measuring lengths and orientations a circle and a chord, respectively, are created on the screen. The circle can be varied in size and position, the chord in direction and position by the operator and so be adjusted to the

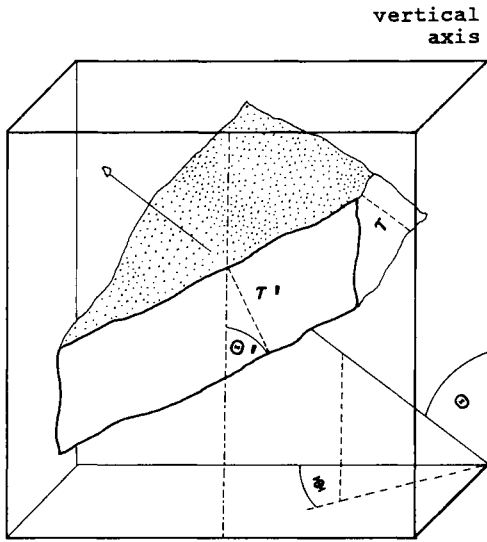


Fig. 5. Geometry of a crack cut by a section plane (front side of the cube). the spatial direction of the normal to the crack indicated by the arrow is defined by Θ and Φ , the true width is τ ; measurements of τ' (apparent width) and Θ' (apparent angle to the vertical axis) can be taken from the profile of the crack visible in the section plane.

section profiles of the pores. The diameter of the circle is recorded as length, the direction of the chord is recorded as the orientation of the pores.

The different pore types are treated as follows:

Channels: The diameter of the largest circle enclosed by the section profile gives “ a ”. The smallest circle, which encloses the section profile gives “ b ”. The chord has to be adjusted to the long diameter of the ellipse, its angle is Θ' (Fig. 2).

Cracks: τ' is measured as the diameter of the circle touching the intersection point and the opposite boundary. For finding Θ' this circle is enlarged by 30% of its diameter; in consequence, the solid-void boundary is cut at two points by this circle. These two points are connected by the chord, its angle is Θ' . (This procedure assures an unambiguous measurement of the orientation which is, moreover, rather insensitive to the surface roughness of the crack boundary, see Fig. 3.)

The evaluation of 1 cm^2 takes 5–15 minutes.

STEREOLOGICAL CALCULATIONS

Channels

The estimation of the joined distribution of widths and orientations in 3D is based on two assumptions, namely that channels are circular in cross sec-

tion and, as pointed out above, that there is rotation symmetry with respect to the vertical axis.

The geometric relationships are shown in Fig. 4. The real width of a channel (a) can be seen in the section, the real orientation is unambiguously defined by the two angles Θ and Φ . The latter is equally distributed in the case of rotation symmetry. According to Sandau (1985) the real angle Θ can be calculated:

$$\Theta = \arcsin((r^2 + (1 - r^2) \times \sin \Theta')^{1/2})$$

where $r = a/b$.

The measurements of a , b and Θ' as described above lead to a two-dimensional frequency table of i classes of widths (a) and j classes of angles (Θ) with the frequencies I_{ij} in each class (i, j) (Table 1) and the total number I_{00} according to the number of profiles measured. The relative frequencies of the classes (i, j) are:

$$r_{ij} = I_{ij}/I_{00}$$

These r_{ij} are estimators for the probabilities $\hat{p}_{ij}$ of the classes (i, j). However, these probabilities depend on a_i and Θ_j , since the probability of hitting a channel by the testlines depends on a_i and Θ_j . In order to calculate these probabilities, vertical and horizontal testlines have to be treated separately. In the following the calculations concerning horizontal testlines are given in brackets:

The probability of hitting a channel of width " a " and orientation Θ and Φ with a vertical [horizontal] testline is proportional to the projection area A_p of this channel on a horizontal [vertical] plane which is given by:

$$A_p = a \times \sin \Theta$$

$$[A_p = a \times (\cos^2 \Phi \times \sin^2 \Theta + \cos^2 \Theta)^{1/2}]$$

The joined distribution of widths and orientation in 3D can be obtained approximately by:

$$p_{ij} = k \times \hat{p}_{ij} / (a_i \times s_j)$$

where k is given by $\sum \sum p_{ij} = 1$, and $a_i \times s_j$ represents the mean projection area on a horizontal [vertical] plane with width a_i and length s_j within each class (i, j). As representative values for each class (i, j) we take the mean values:

$$a_i = (a_{\min} + a_{\max})/2$$

$$[a_i = (a_{\min} + a_{\max})/2]$$

and:

TABLE 1
Frequencies I_{ij} and derived probabilities p_{ij} of i classes of widths and j classes of directions obtained from measurements at vertical testlines. For further explanation see text

Orientation, <i>j</i> classes (°)	<i>S_j</i>	Width, <i>i</i> classes represented by <i>a_i</i> (μm)															
		45		90		160		250		400		625		875		1500	
		<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>	<i>I_{ij}</i>	<i>p_{ij}</i>
0–10	0.087	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10–20	0.258	0	1	0.037	0	0	0	0	0	0	0	0	0	0	0	0	0
20–30	0.422	0	1	0.023	2	0.026	2	0.016	2	0.010	0	1	0.002	0	0.002	0	0
30–40	0.573	0	0	4	0.038	6	0.036	2	0.008	1	0.002	2	0.003	0	0.003	0	0
40–50	0.706	0	1	0.014	11	0.084	5	0.025	7	0.021	5	0.003	1	0.001	0	0.001	0
50–60	0.818	1	0.024	4	0.047	12	0.079	4	0.017	4	0.011	2	0.002	1	0.001	0	0
60–70	0.905	0	3	0.032	13	0.078	4	0.015	9	0.022	1	0.002	1	0.001	0	0.001	0
70–80	0.965	0	3	7	0.039	6	0.022	6	0.013	3	0.004	1	0.001	0	0.001	0	0
80–90	0.995	0	6	12	0.065	10	0.035	18	0.039	2	0.003	0	1	0.001	0	0.001	0

$$s_j = \int_{j_{\min}}^{j_{\max}} \sin \theta_j d\theta$$

$$[s_j = \int_0^{\pi/2} \int_{j_{\min}}^{j_{\max}} (\cos^2 \phi_j \times \sin^2 \theta_j + \cos^2 \theta_j)^{1/2} d\theta d\phi]$$

where the subscripts “min” and “max” indicate the border values of each class (i, j).

Moreover, estimations of the length density L_v (cm/cm^3), the surface density S_v (cm^2/cm^3) and the volume density V_v (cm^3/cm^3) for the channels can be deduced. The determination of the density of intersections I_L on vertical testlines (number of intersections per length of the testline, $1/\text{cm}$) is simple. In general the relation is valid that:

$$I_L = \text{projected area/volume}$$

then for each class (i, j):

$$I_L(i, j) = (l \times a_i \times s_j) / V$$

where l corresponds to the length of the channel. So, the length density of each class (i, j) can be derived as:

$$L_v(i, j) = I_L(i, j) / (a_i \times s_j)$$

and the total length density as $L_v = \sum \sum L_v(i, j)$.

The surface of a channel is $S = l \times \pi \times a$. Consequently, the surface density of each class (i, j) is obtained as:

$$S_v(i, j) = I_L(i, j) \times \pi / s_j$$

and the total surface density as $S_v = \sum \sum S_v(i, j)$.

The volume of a channel is $V = l \times \pi / 4 \times a^2$. Consequently the volume density of each class (i, j) is obtained as:

$$V_v(i, j) = I_L(i, j) \times a_i \times \pi / (4 \times s_j)$$

and the total volume density as $V_v = \sum \sum V_v(i, j)$.

The calculations using vertical and horizontal testlines lead to two independent estimations of the described distributions.

Cracks

For estimating the distribution of orientations and widths of cracks, again rotation symmetry with respect to the vertical axis is assumed.

The geometric relationships are shown in Fig. 5. According to the assump-



Fig. 6. Vertical section of the Bt-horizon of a Luvisol from loess, arable land, northwestern Germany, depth 75–77 cm; voids are black.

tion, the angle Φ is equally distributed. From Fig. 5 it is obvious that for individual profiles τ and Θ cannot be calculated from τ' and Θ' , the parameters measured. According to Sandau (1993) the distribution of τ and Θ can be estimated from combined measurements of τ' and Θ' at a number of profiles. This method is based on assumptions concerning the kind of distribution of τ and Θ .

For the widths τ a lognormal distribution is assumed with mean value $\mu(\Theta)$ and variance σ , where $\mu(\Theta)$ can be dependent on the orientation, since it is not imperative that there is independence between width and orientation of the cracks.

For the angle Θ a small circle distribution according to Bingham and Mardia (Batschelet, 1981) is assumed, which is suitable for the parametric description of orientation distributions showing one preferred orientation. The parameters are Θ_0 for the position of the preferred orientation and k , describing the strength of the preferred orientation.

Combined measurements of τ' and Θ' lead to a two-dimensional frequency

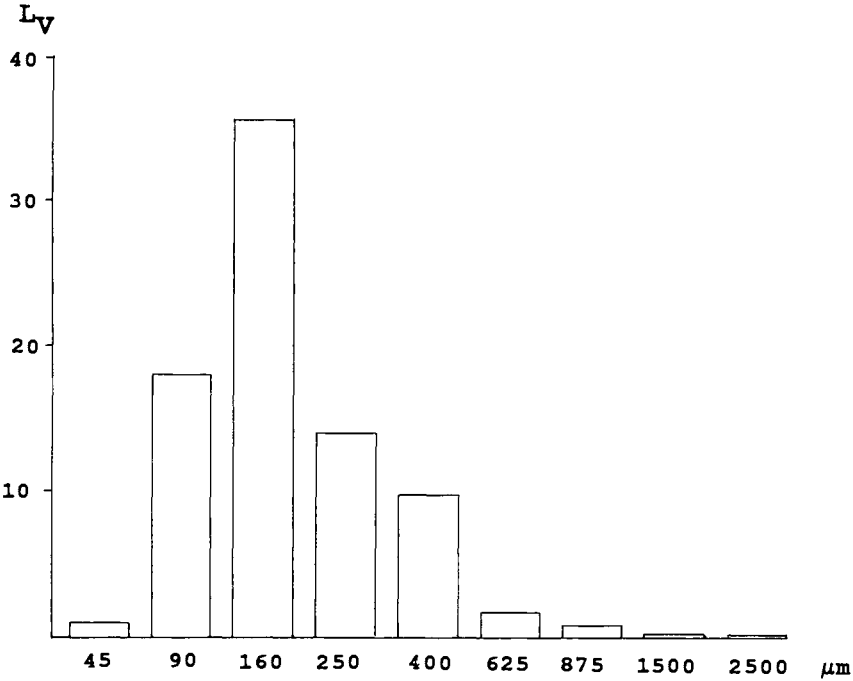


Fig. 7. Length densities L_V (cm/cm³) of channels with different widths (on the abscissa the width classes are plotted with their mean value). Number of profiles measured, $n=289$.

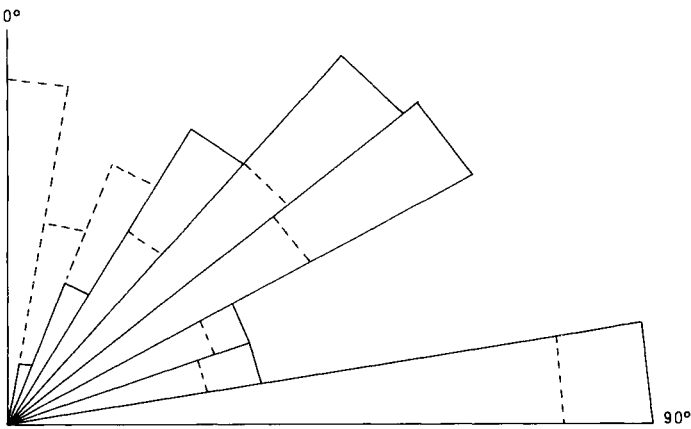


Fig. 8. Orientation distribution of channels in 2D (Θ' , dotted lines) and derived for 3D (Θ), $n=289$.

table with n classes of widths and m classes of orientations, therefore $n \times m$ classes with the frequencies $I(i,j)$. According to Sandau (1992) each parameter vector $\nu(\mu(\Theta), \sigma, \Theta_0, k)$ leads to the probability p_{ij} for each class within

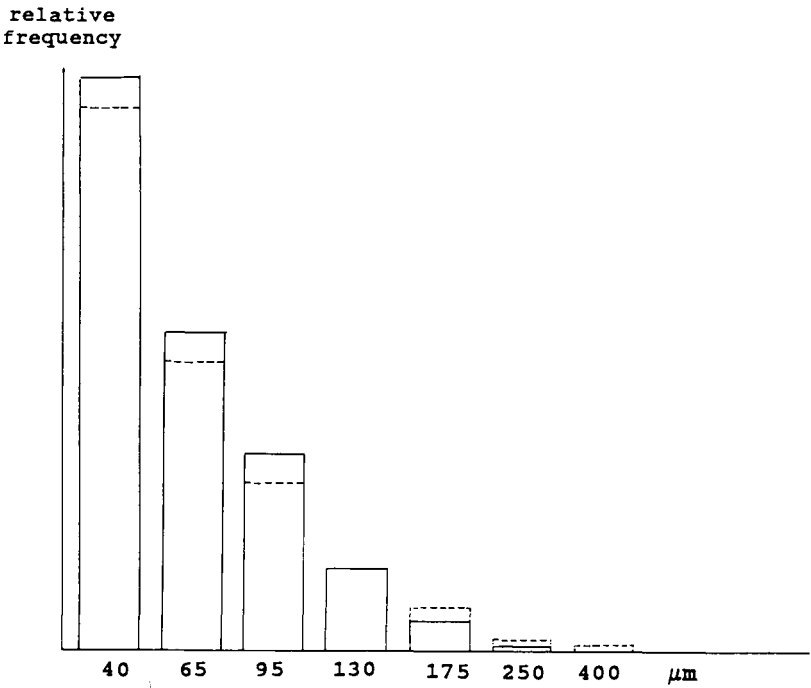


Fig. 9. Width distribution of cracks in 2D (τ' , dotted lines) and derived for 3D (τ), on the abscissa the width classes are plotted with their mean value, $n=227$.

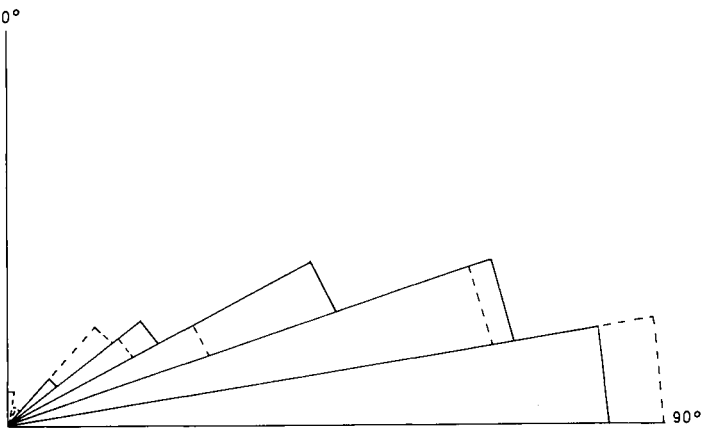


Fig. 10. Orientation distribution of cracks in 2D (θ' , dotted lines) and derived for 3D ($90^\circ - \theta$), $n=227$.

this frequency table. The true parameters are estimated, using the chi-square goodness of fit method. By that, it is also possible to test whether the distributions assumed are adequate for the investigated kind of structure. A computer program for the calculations written in Pascal is available. An example is given by Sandau and Vogel (1993).

EXAMPLE

As an example the results of a Bt horizon of a Luvisol from loess are shown in Figs. 6–10. Within three polished blocks, six areas (2×2 cm) have been evaluated, altogether 24 cm².

The width distribution of channels shows a significant maximum at 150–200 μ m (Fig. 7). This corresponds to a frequently observed diameter of fine roots of herbaceous plants. The orientation distribution of channels shows two different preferred orientations, one at about 45°, the other one at 90° to the vertical axis. (The interpretation is not the object of this paper. But it will have to be discussed to what extent the roots follow endogenous directions, to what extent however they follow the soil structure—e.g., orientation relative to the cracks.)

The true width distribution of cracks is slightly shifted towards lower values than the 2D width (Fig. 9). The same can be said for the orientation distribution in 2D and in 3D (Fig. 10). In 2D the orientations are slightly shifted towards larger angles to the vertical axis. Such insignificant differences have to be expected from a strongly marked preferred orientation perpendicular to the section plane.

DISCUSSION

The prerequisites of the presented methods seem to be fulfilled in most soils to a sufficiently high degree (circular cross section of channels, rotation symmetry of channels and cracks with respect to the vertical axis). The additional assumptions which are necessary for the estimation of the orientation and width distribution of cracks may be more problematic. These are the hypothesis of a lognormal distribution of the widths of cracks and, above all, the small circle distribution with only one maximum for their orientation. The fact that cracks often develop vertical to each other will often cause distributions with two maxima. Nevertheless, the presented procedure may be adopted also in such cases as the calculations can be based on two overlapping small circle distributions. In the presented example the mathematical investigations have proved that the assumption of a distribution with one maximum was justified.

The image analysis as done here may be subject to another criticism, that of subjective error. But an error in the discrimination of channels and cracks

may be no more important than objective errors of automatic image analysis. The procedure produces quite quickly a basis of data which is sufficient for the stereological calculations.

Information about orientation distribution provides a better basis for the calculation of surface densities of channels and cracks and of length densities of channels. In addition, this information may help in qualitative and perhaps quantitative understanding of the anisotropy of functional properties of soil structure. It is true, however, that important information is still lacking, concerning the continuous extension of individual channels or cracks and the spatial connectivity of the pore system. But some cautious assumptions about these may also be made on the basis of orientation distribution and surface densities of channels and cracks.

The example shows that even the qualitative ideas about orientation and width distribution usually are biased when based on the 2D image only. It shows also that quantitative data on orientations make possible more specific discussions on the formation of pore systems.

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Measurement of proliferation and biomass of fungal hyphae and roots

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ABSTRACT

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New questions are being asked about the functional role of soil fungi, plant roots and their interactions. Existing methods to study the species composition and biomass of soil fungi and roots are outlined and the limitations of the data derived from them are discussed. The integration of these existing methods and new technologies in multi-disciplinary studies are proposed as the way forward to adding measures of function to those of biomass.

INTRODUCTION

This paper is intended to outline some of the methods available for the study of fungal hyphae and root growth in soil and to ask if they are adequate to enable us to understand the functioning of these components in the soil ecosystem or to understand the effects of perturbations on the functions of these components. As such it is a document designed to initiate discussion in the workshop forum, rather than being a comprehensive review.

In soil, saprotrophic fungi degrade organic resources and, by use of the carbon material contained therein, mineralise inorganic nutrients which are then available for uptake by plant roots and their associated mycorrhizal fungi. The proliferation of hyphae and roots, therefore, tends to be related to the spatial heterogeneity found in soil. Differences in physiological capabilities of different fungal species [some capable of cellulose and/or lignin degradation whilst others are not (Dickinson and Pugh, 1974)] shows that there is speci-

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alisation within the saprotrophic fungal community. This together with differences in species composition of ectomycorrhizal fungal species with tree age (Last et al., 1987) and the nutrient uptake capacity of different mycorrhizal fungal species (Dighton et al., 1990), suggests that the species composition as well as biomass of the fungal community is an important determinant in the functioning of the fungal components at any one site in the soil. Root system architecture may also be important in the ability of a plant to access nutrients from a heterogeneous environment (Fitter, 1985) and hence the species composition of the rooting components and its mycorrhizal flora also need to be taken into account. The spatial and temporal distribution of fungi and roots, their interactions with other soil organisms are all important factors determining the rates of nutrient cycling processes in soil.

Our present understanding of fungi and roots in soil has come from a number of methods which determine the biomass of these components in soil and their physiology in (usually) monospecific studies in artificial laboratory experimental conditions. Few studies have looked at either interactions between organisms or their physiology in the soil environment. This paper will briefly outline some of the methods currently available for the study of fungi and roots in soil and explore how far these go in terms of understanding the functional interrelationships between organisms in soil.

PROLIFERATION OF FUNGAL HYPHAE

Measures of fungal biomass

Recent reviews by Frankland et al. (1990), Gray (1990) and Parkinson and Coleman (1991) compare the methods available for the assessment of microbial (including) fungal components of soil. An outline of the range of methods is given in Table 1. Direct observation of small volumes of soil can reveal information on the spatial distribution of fungi and allows limited identification of the fungal components on the basis of hyphal morphology (Ponge, 1990). Extraction techniques and dilution plating, soil plates (Warcup, 1950) and soil washing (Parkinson and Williams, 1961) can provide some evidence of the species composition of the soil fungal community, although this is restricted by the limitations of propagule viability (spore germination, hyphal fragment viability) and the selective nature of artificial media on which the isolates are grown. Modified versions of the Jones and Mollison (1948) agar film technique for soil (Parkinson et al., 1971) and litter (Frankland et al., 1978) and the membrane filtration technique (Hansen et al., 1974) allow quantification of fungal hyphal biomass. These methods, however, do not allow species composition to be assessed or to readily distinguish between living and dead mycelia. The use of vital stains, particularly fluorescein diacetate, have gone some way towards differentiating living

TABLE 1

Outline of methods commonly used for the study of fungi in soil

<i>Observation</i>	
Macroscopic features	Fruitbody production distribution
Light microscopy	Soil smears, sections, particles etc, in conjunction with general or vital stains and immunological techniques
Electron microscopy	Soil sections or particles using TEM and SEM
<i>Isolation (identification and biomass determination)</i>	
Traps and baits	Mainly for identification
Hyphal extraction	Biomass
Dilution plating	Biomass (and identification)
Soil plates	Biomass (and identification)
Soil washing	Identification
DNA fingerprinting	Identification
<i>Measures of activity</i>	
Metabolism	CO ₂ production from respiration, ATP production
Enzyme activity	Cellulase, proteinase, phosphatase etc., enzyme production
Utilisation of organic resources	Decomposer ability determined by litter bags, cotton strips, etc

from dead mycelium in soil (Söderström, 1977, 1979). Substrate induced respiration (Anderson and Domsch, 1975, 1978) can also give an index of fungal biomass, but not species composition. However, one of the benefits of this method is its ability to distinguish between fungal and bacterial activity in soil (Beare et al., 1991).

Recent advances in immunological techniques have allowed identification of fungal hyphae in situ to be made using fluorescent antibody techniques (Chard et al., 1985a, b). Antibody techniques, however, require considerable time investment in the production of antigenic proteins. Recent progress in DNA fingerprinting techniques such as PCR (RAPD) (Erlich, 1989; Williams et al., 1991) have the potential to resolve identification to a single diploid cell using crude DNA extracts and random oligonucleotide primers to allow amplification of homologous DNA sequences from the unknown source which are then separated by gel electrophoresis. This technique is already being used to identify ectomycorrhizas on tree root (Gardes et al., 1991). By a combination of techniques, therefore, it is now becoming possible to quantify the contribution of each fungal species to the population.

Interactions between fungi: spatio-temporal distribution

Soil is a very heterogeneous environment and the abundance and distribution of fungal hyphae within this matrix is dependent upon the relation of the physico-chemical heterogeneity of the soil matrix (considered here as the mineral, organic and soil solution components) and the demands for carbon (energy) and nutrients of the fungal community. These physico-chemical

constraints define boundaries of the niche for the fungal community as defined by Swift (1976, 1984). This unit community is a species assemblage inhabiting a clearly delimited resource where the components of the community within the unit interact but interaction between neighbouring unit communities is restricted. Examples of resource units are given as twigs or branches, dead tree boles, faecal pellets and leaves. His discussions (Swift, 1984) likens the distribution of these resources to islands and develops the notion that invasion, colonisation and extinction of fungal communities on these island resources could be interpreted according to classical island biogeography theory (MacArthur and Wilson, 1967). Similarly a niche is defined in terms of resource quality and the development of its unit fungal saprotrophic communities has been elaborated by Cooke and Rayner (1984). Based on the observations of successions of fungi colonising resources (see patterns of change for fungal communities with time on different litters reported in Dickinson and Pugh (1974) and Gourbiere (1990)) and competition between fungi colonising dead wood (Rayner, 1978; Boddy and Rayner, 1983) fungi may be assigned to categories of competitive strategy according to the r-K continuum (MacArthur and Wilson, 1967) and the R-C-S concept of Grime (1979). This is further discussed in context of carbon turnover by microflora by Heal and Ineson (1984). Rayner et al. (1985a) discuss the mechanisms and patterns of colonisation of resources in terms of availability and uptake of nutrients, stimulation and inhibition by chemical factors (recognition), establishment and spread of mycelia, including competition and exit patterns.

It is appreciated that most population models are based on "discrete" organisms. In the case of the fungal thallus, considerable connectivity between resource "islands" occurs and the physico-chemical nature of these "islands" influences the colonisation pattern (rate of growth of the mycelium) within the "island" and growth between "islands" (emigration). It is possible that theories of island biogeography are too simplistic for fungi. However, from an assessment of the competition strategies of different fungal species (or trophic groups), the basic models of island biogeography and patch dynamics theory (White and Pickett, 1985) and the changes in resource quality (niche breadth, sensu Swift, 1976) we can attempt to develop predictive models of the rate of colonisation and species composition of the fungal community developing on individual resource units in the heterogeneous soil environment.

Fungal physiology

Saprotrophic fungi in soil are involved in the decomposition of organic resources. Gross measures of decomposition activity by determination of respired CO₂, lysimetry of mineralised nutrients or gross measures of substrate loss (Harrison et al., 1988) give a measure of fungal and microbial activity at

a macro scale of resolution. The role of fungi in the processes of temperate forest ecosystems (as reviewed by Dighton and Boddy, 1989) involve spatio-temporal activities at lower levels of resolution, where we may consider the activities of individual fungal hyphae or the fungal thallus as a unit. In order to do this we need to know where the hyphae of the particular fungus is in a spatial context, its linkages with other parts of the thallus and its physiological activity. The fungal thallus is not a discrete unit as are soil animals, but a diffuse continuum whose physiology can differ in different parts of the thallus; for example, an active hyphal front may act only as a sink for carbohydrates and nutrients involved in biomass production, active uptake of carbon and nutrients may occur behind this front where enzyme activity is greatest, whereas other regions may be dying and lysing with either the loss or retranslocation of nutrients and carbon to other parts of the thallus (Fig. 1). Rayner et al. (1985b) discuss the development of the fungal thallus into linear organs (rhizomorphs) and the controls on growth producing "slow dense" or "fast diffuse" hyphal development. The function of these linear organs or rhizomorphs as nutrient translocatory organs has been investigated in the laboratory and field (Brownlee and Jennings, 1982; Clipson et al., 1987; Cairney et

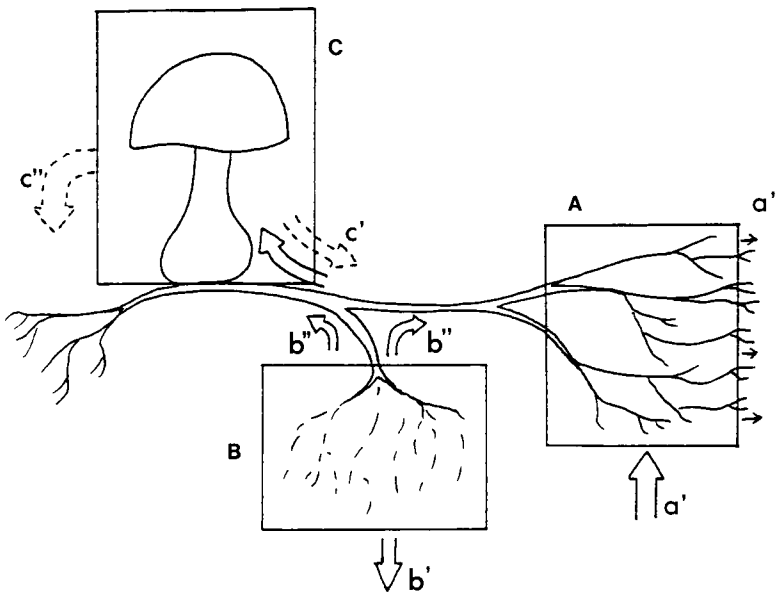


Fig. 1. Gains, losses and potential directional translocation of nutrients in fungal thalli. Box A shows consolidated hyphae absorbing carbon and nutrients from organic material (a') and movement of resources to new adventitious hyphae (a''). Box B shows loss (b') or conservation and retranslocation (b'') of resources from dying and lysing mycelia. Box C shows directional translocation to initiate fructification (solid arrow) and conservation and retranslocation (c') or loss of resource (c'') on senescence of the structure.

al., 1988; Wells and Boddy, 1990; Wells et al., 1990). Translocation within diffuse mycelial thalli and directional transport within thalli between changing source/sink strengths (Fig. 1) have not been so thoroughly explored and may be important at smaller scales of resolution. However, Girvin and Thain (1987) and Thain and Girvin (1987) have demonstrated translocation of ^{86}Rb in mycelial thalli of *Neurospora crassa* at rates greater than mycelial extension rates, in laboratory conditions. In this context, the use of both stable and radioisotope tracer techniques could be usefully employed in the field.

With respect to detecting localised zones of enzyme production, it is possible that fungi could be genetically manipulated by placing LUX genes in combination with those producing selective enzymes. Microsites of enzyme activity could then be detected by luminescence of the fungal hyphae during the period of gene expression.

PROLIFERATION OF ROOTS

Measurement of root biomass

As for fungi, the methods used for evaluation of root growth and biomass vary depending on the scale of resolution required by the worker. Many of the methods available for root biomass determination and root observation are explored in the volume edited by Atkinson (1991) and summarised in Table 2. Harper et al. (1991) demonstrate the problems of getting to grips with the dynamics of root growth and development in an opaque medium, concluding that "Plant root systems present the research worker with many of the greatest unsolved problems in the plant sciences. Their evolution, development, form and function are all sources of open questions that demand new technologies and imaginative insights".

TABLE 2

Outline of methods commonly used for the study of root dynamics

<i>Destructive sampling</i>	
Whole root excavations	Usually limited recovery of fine root fraction
Repeated coring and extraction	Mainly for fine root fraction, problems of recovery of necromass
Root ingrowth cores	Differences in soil structure and composition within and without ingrowth core
<i>Direct observation</i>	
Rhizotrons	-Large scale field; able to measure replicate plants in 2D space -Mini- and micro-rhizotrons: replication of small volumes of soil throughout treatment plot -Laboratory; artificial soil structure

These techniques have led to development of image analysis methods to aid measurement of root growth.

At the macro scale of resolution, root biomass is usually estimated by either whole root excavation, or by coring and extraction of roots. Biomass and root turnover can be assessed from replicate core samples and replicates taken at repeated time intervals. This, and other methods relating to forest trees are reviewed by Persson (1989). At smaller scales of resolution, direct observation techniques are used, employing root observation laboratory (rhizotrons) or mini- or micro-rhizotrons, where small volumes of soil may be observed *in situ* using boroscopes. These techniques are discussed in Atkinson (1991) and J. Lussenhop's paper in this special issue where the problems of root length/biomass assessment of the different techniques are compared. These root observation techniques allow more detailed data to be obtained regarding the spatio-temporal distribution of roots and root growth and allow indices of root turnover to be made by observation rather than by estimating differences in biomass from sequential root coring (Cheng et al., 1991) although adequate calibration of measures in 2D space to extrapolation to 3D space need further study. The effect of a planar surface at the glass window/soil interface, may, however, affect the microsite physico-chemical conditions compared to the bulk soil, so that the data derived from these studies must be interpreted with care. Therefore, there is value in looking at a range of systems from a totally artificial soil or level of compaction and structure to give broad ideas of root behaviour and growth rate at one end of the spectrum and minimal disturbance of soil profiles at the other end of the spectrum, to get as close to the real world as possible.

The rhizotron methods are in their infancy and it is recognized that more information will be gained in their suitability as a research tool by their continued use.

The distribution of a root system of a plant in the soil can be mapped or assessed by excavations of small root systems. Study of the architecture of the root system, in terms of root length, frequency and angle of branching have allowed Fitter (1985) to develop mathematical formulae to describe root architecture and to develop computer simulations of their growth. From these models he has made cost/benefit analyses in terms of carbon required to support root growth in relation to nutrient acquisition and anchorage of the plant (Fitter, 1991). An additional feature affecting the spatial distribution of roots, however, is the plastic response to heterogeneity of nutrient distribution in soil. Roots proliferate in nutrient-rich zones (Rogers and Head, 1969; Drew et al., 1973) and fine ectomycorrhizal roots of forest trees are most abundant in the lower organic horizons and well decayed woody debris (Harvey et al., 1979) where nutrient mineralization is occurring. Concentrated growth of roots in levels of high nutrient availability have been elegantly demonstrated in laboratory studies by Grime et al. (1991) where root proliferation was increased in zones of high nutritional status in sand culture.

Mycorrhizas

In many instances the interaction between plant roots and fungi need to be considered in relation to exploitation of soil. Many plant species have mycorrhizal associations and, in this situation, it is the fungal component of the symbiont which appears to function largely in the acquisition of nutrients from the soil. Growth of ectomycorrhizal hyphae into soil may be similar to that of saprotrophic fungi, consisting of "fast diffuse" and "slow dense" components, often forming rhizomorphs (Read et al., 1985). Mycorrhizal hyphae may, like roots, proliferate in sites of nutrient mineralization around pockets of organic matter in mineral soil (St John et al., 1983) and, through the excretion of enzymes. Ectomycorrhizal hyphae may be involved in the breakdown of organic matter to release mineral nutrients (see review by Dighton, 1991). The spatial relationship between plant roots, mycorrhizal and saprotrophic fungal hyphae may be important in this context. Janos (1983) suggests that in tropical systems it is the close juxtaposition between mycorrhizal roots and sites of mineralization in organic matter that accounts for efficient nutrient sequestering by plants.

The intimate relationships between extramatrical hyphae of mycorrhizal fungi and saprotrophic fungi in situ have not been fully explored either in a descriptive or functional context because of the difficulties in distinguishing the hyphae. However, from laboratory studies, it seems that their interactions may significantly affect nutrient acquisition by plants (Dighton et al., 1987). Ectomycorrhizal associations with roots can also influence root architecture in terms of altering relative rates of root growth and changing internal link lengths. This factor has been explored in terms of competition between mycorrhizal fungi to colonise roots and the outcomes of interactions in microcosms have led to the development of simulation models of root colonisation (Shaw, 1991). Similarly, the effects of VAM infection on root architecture has also been discussed by Hetrick (1991).

One of the challenges of mycorrhizal work is the study of extramatrical hyphae. The increased advantage of a mycorrhizal over a non-mycorrhizal root is assumed to be the ability of the mycorrhizal fungi to exploit a larger volume of soil thus to extend nutrient depletion zones (Nye and Tinker, 1977; Robinson, 1991) further from the root surface at low energy expense to the host plant. Much of the physiology of mycorrhizas has been carried out using excised roots in which the hyphal connections to soil have been severed. It is only recently that measures of extramatrical hyphal biomass have been made (Bethlenfalvay and Ames, 1987) and methods developed to partition the function of root surface and mycorrhizal hyphae in the uptake of nutrients (Faber et al., 1991) under laboratory conditions. The use of radioisotope tracers (Dighton et al., 1990) shows potential for studies in the field and could be adapted for the study of extramatrical hyphae. These studies are essential

for the complete understanding of mycorrhizal function as quoted by Allen (1991). "We know little regarding the rates and extent of material flows through hyphae and independence of action of the various hyphal segments that make up that mycelium..... Thus, the degree of spatial and temporal separation among the various mycelial networks comprising the mycorrhizae in a community is important in describing the activity of mycorrhizae".

DISCUSSION — METHODS IN CONTEXT

The methods to be adopted for any one study must relate to the question being posed by the study. These questions will determine the scale of resolution at which the study will be conducted and the use of Hierarchical Theory (O'Neill et al., 1991) can be used to prioritize areas of research at different scales of resolution.

Many previous studies of fungi and roots have looked at gross effects, measuring biomass and species composition of the community. The limitations of techniques to isolate and culture fungi mean that those recovered by these methods may only represent a fraction of the total population in soil. Many of the important interactions between roots, fungi and soil occur at the micro scale; such as their role in formation of soil aggregates (Reid and Goss, 1981; Tisdall and Oades, 1982) or in the decomposition of individual organic resources.

In many respects the growth and proliferation of fungal hyphae and roots can be regarded as being similar (Fig. 2) and estimates of rates and pattern of growth of each species of fungus and root in the system under study will have to be determined. Modern computing techniques to aid image analysis (Morgan et al., 1991) will be useful in measuring biomass and growth. Calculation of fractal indices is a potential tool for measuring space exploration, connectivity, complexity and scaling rates for extrapolation within hierarchical levels (Sugihara and May, 1990). These methods can be linked to new molecular techniques such as DNA fingerprinting to determine species composition and in situ measures of activity such as fluxes of radiotracers or identification of enzyme expression. Thus the functional relationships between components in the fungal biomass and roots in soil may be elucidated at a micro scale of resolution. This type of study will be a useful adjunct to the current studies measuring biomass and species composition of populations in the field and the physiology of individual species in controlled conditions at either end of the spectrum. More integrated studies to determine the function of fungi and roots in the "real world" will require close cooperation between researchers of many different disciplines to overcome the problems of working in such a complex environment.

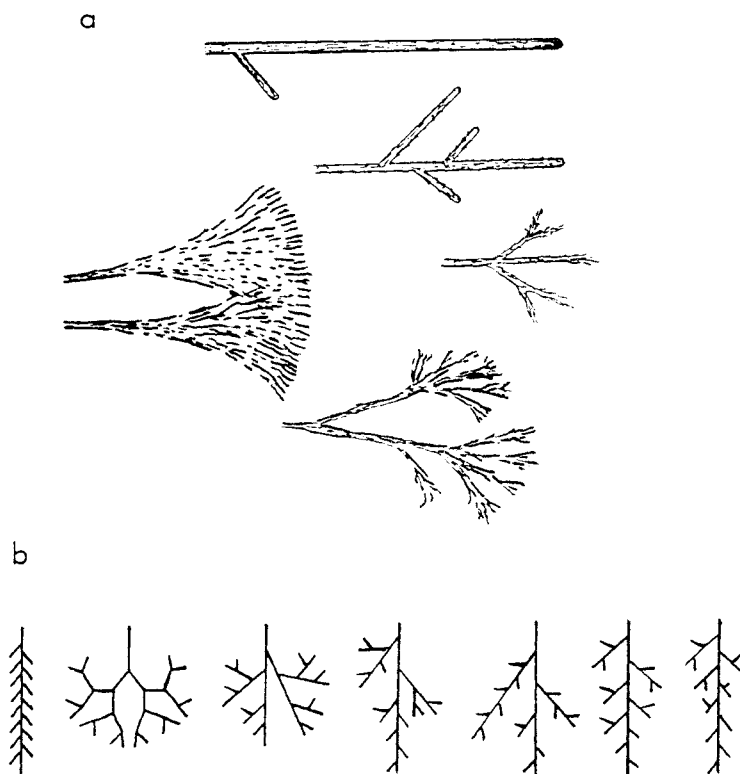


Fig. 2. Diagram indicating (a) ranges in level of organisation of linear mycelial organs and (b) variable tree root architecture. These may be influenced by soil heterogeneity. Is this a case where fractal dimensions may give a mathematical index to describe form? (Modified from Rayner, 1985 and Fitter, 1985.)

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Biological and physico-chemical processes in excrements of soil animals

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ABSTRACT

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Soil animals, especially saprophagous species, ingest large amounts of organic and/or mineral materials. The undigested material is voided as excrements, which are physical structures with specific microenvironmental conditions that may dramatically influence the biological and physico-chemical processes as compared to those occurring in the initial material. In this paper, we emphasize firstly some critical constraints that have to be taken into account in studies of processes in soil fauna excrements, such as (i) the diversity in composition, size and stability of excrements, (ii) the difficulty in sampling excrements in field studies, and/or (iii) the difficulty in defining a relevant reference material for comparative approaches, and (iv) a relevant time-scale for dynamic studies. A review of recent literature enlightens some specific aspects of biological processes in soil fauna excrements, e.g., the composition of the microbial community, microbial activity, decomposition and nutrient release in addition to specific physico-chemical conditions, e.g., aggregation, clay/organic matter interactions and fluid movements. This synthesis emphasizes the close relationship between physical and biological processes that need to be investigated through interactive approaches.

INTRODUCTION

In many ecosystems, the saprophagous soil fauna ingest either pure organic substances (litter, plant debris) or a mixture of organic and mineral material. Because of the low nutritive value of much of the food material, they have to ingest large amounts (Table 1), and as a consequence of their low assimilation efficiency they produce large amounts of undigested material in the form of excrements. In some ecosystems, the structural aspect of the soil profile even suggests that the upper layers might exclusively consist of fresh and old

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TABLE 1

Ingestion rate of different taxinomic groups of soil fauna, in contrasted ecosystems

Taxonomic group	Soil/vegetation	Ingestion rate		Reference
		litter (kg ha ⁻¹)	soil (t ha ⁻¹)	
Termites	Guinean savanna (Niger)	900		Collins (1981)
Enchytraeidae	Arable field (Germany)		0.75	Didden (1990)
Earthworms	Temperate pastures (France)		40–70	Bouché (1982)
Earthworms	Humid savanna (Ivory Coast)	180	800–1200	Lavelle (1978)

excrements (Rusek, 1975; Blanchart, 1990; Dawod and Fitzpatrick, 1993). These excrements are physical structures offering micro-environmental conditions very different from those occurring in the original material, resulting from both digestion processes and intrinsic characteristics. Digestion processes are generally associated with tremendous modifications of the initial material such as:

- fragmentation of coarse organic particles (Rusek, 1985; A. Martin, 1991);
- modification of microbial communities and activities (Parle, 1963a);
- modification of microbial enzymatic digestion, leading to nutrient release (Lavelle and Martin, 1992);
- alteration of the soil structure in different ways (aggregation pattern, water and air movement) (Barois et al., 1993; Blanchart et al., 1993).

This paper focuses on the question to what extent these biological and physico-chemical processes are affected by the specific microenvironmental conditions occurring in excrements, referring to recent studies based on relevant methodologies. The aim is to discuss the state of the art of the knowledge and to discuss strategies for future research.

Methodologies used for studying the processes in excrements are generally derived from routine soil science techniques, but frequently require relevant adaptations due to specific constraints. The major constraints to be considered for such studies are:

- (1) The wide diversity of excrements, resulting from the wide diversity of soil animals with regard to their habitat, diet, and size. The wide range in the composition of excrements directly reflects the wide range of the diet of soil fauna that varies from pure soil organic substances (litter, plant debris) to almost pure mineral material. The flexibility of soil animals in food choice (Anderson, 1983) implies a high variability in their casts too, depending on seasonal changes and habitat. The wide size range of casts is related to the

variety of body size in soil animals: collembola excrements measure less than one millimetre, while earthworm casts may reach several centimetres. For some analyses new microtechniques have to be developed to allow measurements on very small samples.

(2) The sampling of excrements. Sampling does not present any difficulty for animals that survive very well under laboratory conditions, e.g., earthworms, macro- and micro-arthropods. However, for other groups, sampling of excrements may be much more difficult. Termites are a good example, because they do not survive very well under laboratory conditions and also because termite mounds generally result from the accumulation of oral and faecal material in pellets of different ages; these pellets are also sometimes formed into a homogeneous structure, making individual pellets difficult to distinguish or date. A relevant sampling of excrements, particularly in the field, thus requires precise identification of specific origin and age.

(3) The definition of a reference in comparative approaches. Comparative approaches, investigating to what extent processes occurring in excrements are different from those occurring in the undigested material, raise the critical problem of "reference material". In such studies, a precise identification of the material ingested by the animals is required, which is particularly difficult for species feeding selectively on a specific material within the soil matrix. For example, A. Martin (1989) described the difficulty in comparing the biological processes occurring in the soil and in casts produced by a polyhumic geophagous earthworm living in the upper layers of the soil and feeding selectively on organic particles of the soil, because it was impossible to establish what the worm really ingests.

(4) The definition of a realistic time-scale of study. Excrements are temporary physical units that may be destroyed by erosion or coprophagous species within a few hours (e.g., earthworm surface casts, woodlice pellets) or months (e.g. earthworms casts deposited in the soil profile). In order to determine to what extent biological and physico-chemical processes are affected by the specific microenvironmental conditions occurring in excrements, a precise definition of the period of time during which an excrement remains a physical entity, before being recycled into soil or litter, is required. This parameter, which may be sometimes rather difficult to evaluate in natural conditions, is of primary importance to define the time scale of study.

Many studies on excrements of soil fauna in the soil profile are based on micromorphological observations (e.g., Jongerius and Schelling, 1960; Babel, 1975; Rusek, 1975; Mermut, 1985; Pawluk, 1985). These studies have resulted in a wide knowledge on shape of fresh and ageing casts. There appear to be differences in stability of different types of excrements (although of course the lack of morphological changes does not necessarily implicate that no changes in (biochemical) processes have occurred during the ageing). The micromorphological investigations show that there are aspects of the shape of

excrements that impose constraints on the biological and physical processes that take place in the excrements.

After the morphological description, this paper will successively deal with some biological and physical processes, which are expected to be strongly affected by the specific microenvironmental conditions occurring in excrements, i.e.:

I. Biological processes:

- (a) composition of microbial communities;
- (b) microbial activity;
- (c) decomposition and nutrient release.

II. Physical processes

- (a) aggregation, related to clay/organic matter interactions;
- (b) Gas and fluid movement, related to porosity.

These processes may be closely interdependent, i.e. microbial activity and oxygen diffusion, decomposition and organic matter/clay interactions, etc. For that reason, particular attention will be paid to studies investigating biological and physical processes as interactive processes.

MORPHOLOGICAL STUDIES

Excrements of a wide variety of animals of different species and size have been micromorphologically described in many papers. Also information is available on the pattern of ageing of excrements in their natural environment. Some excrements, like those of many species of Oribatei are very compact (Rusek, 1975) and do not change much over time (Bal, 1970; Dinc et al., 1976). After due time, they fall apart to small bits of humus material that can form the humus found in the mineral layer of podzols (Bal, 1970). Other excrements slowly disintegrate, thereby losing their characteristic shape more and more, until they are no longer recognizable as excrements. Examples of this are given by Bal (1973). Many excrements in the profile do not disappear by ageing but because of coprophagic activities of other soil animals. Babel (1975) described the destruction of enchytraeid droppings by earthworms, while some organisms such as woodlice or earthworms can reingest their own excrements or feed on the excrements of other organisms (Gunnarson and Tunlid, 1986). Excrements of enchytraeids and collembola are eaten by Diptera larvae according to Bal (1970).

The relative stability of some excrements may be ascribed to the presence of a peritrophic membrane (Bal, 1970, 1973) or a compact outer coating of the excrement (Blanchart, 1993). Other excrements are stable because they are very compact. This can occur both in organic or organo-mineral excrements (Bal, 1973).

BIOLOGICAL PROCESSES IN EXCREMENTS OF SOIL FAUNA

Biological processes in soil are mainly accounted for by microbial activity involved in decomposition of organic matter. Investigation of microbial processes should include the composition of the microbial community, measurements of the microbial activity, e.g. enzymatic activities, and release or uptake of gases (CO_2 , O_2 , N_2) and nutrients (N, P, K, ...).

Composition of the microbial community

Many studies, based on classical microbiological techniques such as direct isolation (fungi), sequential washing, plate counting and diagnostic tests (see the methodological review in Parkinson and Coleman, 1991), indicate that the microbial community shows a specific pattern in excrements of soil animals, as a result of the digestion processes and the specific microenvironmental conditions which occur in excrements (pH, oxygen status, water content, ...). An enrichment of bacteria and actinomycetes in fresh excrements, in comparison to litter or soil was observed in a wide range of different taxonomic groups such as woodlice (Hanlon and Anderson, 1980; Ineson and Anderson, 1985; Gunnarson and Tunlid, 1986; Ullrich et al., 1991), millipedes (Anderson and Bignell, 1980; Hanlon and Anderson, 1980; Ineson and Anderson, 1985) and earthworms (Day, 1950; Parle, 1963b; Kozlovskaya, 1969). The abundance of bacteria and actinomycetes in fresh casts of earthworms is considered to directly result from the conditions prevailing in the gut, which are more suitable for growth of bacteria than of yeasts and fungi (Parle, 1963a). Moreover, a specific bacterial community may occur in fresh excrements: In a microcosm study Ullrich et al. (1991) observed that the

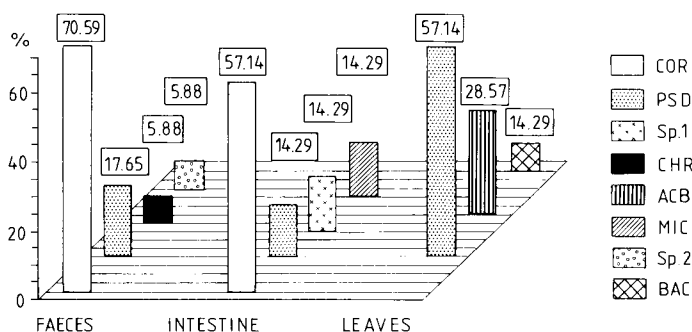


Fig. 1. Composition of the bacterial microflora in faeces and intestine of *Oniscus asellus* (Crustacea, Isopoda) as compared to that of partially decomposed leaves of beech, oak and maple (from Ullrich et al., 1991). COR = coryneform bacteria, PSD = pseudomonas, Sp.1 = not specified species No. 1, CHR = *Chromobacterium violaceum*, ACB = *Acinobacter calcoaceticus*, MIC = micrococcaceae, Sp.2 = not specified species No. 2, BAC = *Bacillus cereus*.

bacterial community of partly decomposed leaves was dominated by *Pseudomonas* sp. while that of fresh excrements of the woodlouse *Oniscus asellus* was dominated by coryneform bacteria, a difference which might have changed the decomposition processes (Fig. 1). Long-term studies indicate that the microenvironmental conditions occurring in excrements may also influence the succession of microbial communities during ageing of excrements. In a laboratory experiment, Nicholson et al. (1966) observed that the microbial community of fresh faeces of the millipede *Glomeris marginata*, fed on hazel leaf litter, was dominated by bacteria, while that of faeces older than 20 days was dominated by fungi, which developed superficial hyphae (Fig. 2). Such a rapid development of superficial fungal hyphae in ageing excrements has also been observed in enchytraeid faeces (Toutain et al., 1982) and earthworm casts (Marinissen and Dexter, 1990). The phenomenon may enhance the structural stability of these excrements (Marinissen and Dexter, 1990), consequently influencing the physical processes occurring within the aggregates. In order to relate biological and physical processes, more attention should be paid to the quantitative estimation of fungal hyphae in soil animal excrements. These studies may be facilitated by using recent techniques such as fluorescent staining linked to computed image analysis (Morgan et al., 1991).

Conversely, the estimation of microbial biomass in excrements of soil fauna, obtained either by conversion of microbe counting or direct chemical extraction, has provided conflicting results (Scheu, 1987; Mulongoy and Bedoret, 1989; Lavelle and Martin, 1992). It is not yet clear whether this is a matter of methodological differences or that there really exist differences between either different species of soil fauna or even within species under different conditions. This matter needs further investigation.

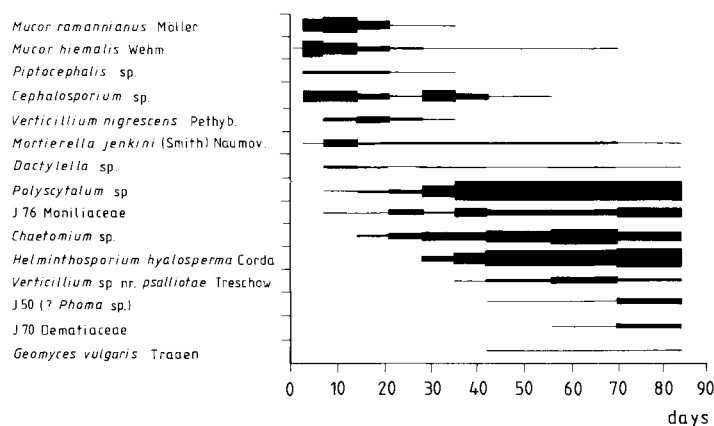


Fig. 2. Succession of fungi on *Glomeris* pellets decomposing in the laboratory. The depth of each histogram is proportional to the percentage of samples colonized. n (100%) = 30. (From Nicholson et al., 1966.)

Microbial activity

Classical methodologies used to investigate microbial activity in soil or litter may be adapted to study the microbial activity in excrements of soil fauna (see the methodological review in Parkinson and Coleman, 1991), for most of these methods, however, it is required that specific microtechnical adaptations are developed when very small samples need to be analyzed.

Enzymatic activity is classically measured by addition of specific substrate followed by incubation (see, e.g. Perez Mateos and Gonzalez Carcedo, 1985). The enzymatic activity in excrements, may provide information about the functioning of the microbial community. Excrements are expected to show specific enzymatic activity, related to a specific microbial community. A strong increase in phosphatase activity has been reported in fresh casts of earthworms from temperate regions (Sharpley and Syers, 1976; Fig. 3), especially when background phosphatase activity in the soil was low (Satchell and Martin, 1984). Mulongoy and Bedoret (1989) also observed a strong enzymatic activity in casts of tropical earthworms, compared to the surrounding soil, for a large range of enzymes, i.e. urease, dehydrogenase, glucosidase and phosphatase. Conversely, no difference in phosphatase activity was observed in a termite mound in comparison with the surrounding soil in a Venezuelan savanna (López-Hernández et al., 1989).

The microbial respiration is directly related to the background metabolic activity of microbes. Many studies, based on the classical Warburg method or on the microrespirometry method, specifically developed for very small samples (Verdier, 1983), point to an increase in CO₂ release from fresh excrements of a wide range of animals, e.g., millipedes (Nicholson et al., 1966),

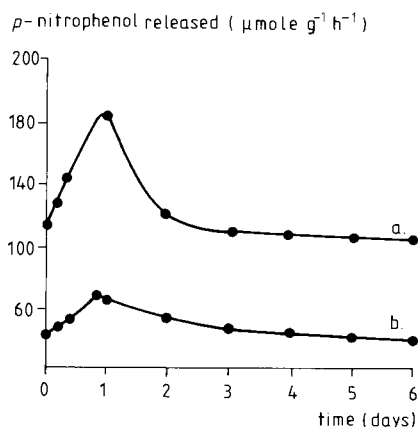


Fig. 3. Phosphatase activity, determined by release of *p*-nitrophenol, as a function of time of incubation at 16°C of fresh casts of *Lumbricus rubellus* and *Aporrectodea caliginosa* (a) and underlying surface soil (0–5 cm) (b). (From Syers and Springett, 1984.)

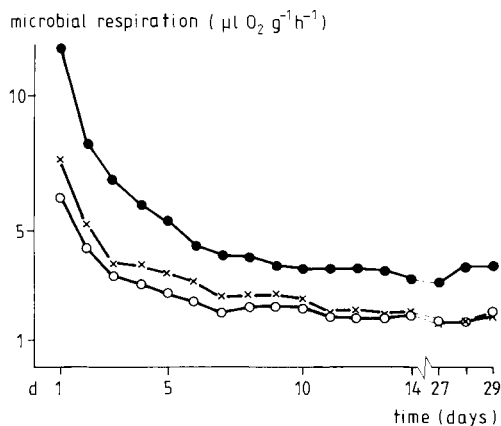


Fig. 4. Changes in time in microbial respiration in soil ($\times$), burrow walls ($\circ$), and faeces ($\bullet$) of earthworms (*Aporrectodea caliginosa*). (From Scheu, 1987.)

woodlice and earthworms (Barois and Lavelle, 1986; Scheu, 1987; Elliott et al., 1991), as compared to litter and soil. The microbial activation in fresh excrements might result from (i) an increase in pH that may occur during gut transit (Barois and Lavelle, 1986; Lal, 1988; Mulongoy and Bedoret, 1989), (ii) the release of large amounts of water soluble organic components during gut transit (Martin et al., 1987), or (iii) comminution which enhances organic matter availability for microbes (S. Martin, 1991). However, microbial activation is often temporary and decreases rapidly in ageing excrements (Nicholson et al., 1966; Barois and Lavelle, 1986; Scheu, 1987) (Fig. 4).

Nutrient release and decomposition

The enhancement of microbial (enzyme) activity in fresh excrements may result in release of nutrients. Satchell and Martin (1984) reported high inorganic P content in fresh earthworm casts as a result of high phosphatase activity. However, there is no clear relationship between nutrient release and enzymatic activity: López-Hernández et al. (1989) measured large amounts of inorganic P in termite mounds from Venezuelan savannas, although phosphatase activity was very low. High nutrient content in fresh excrements may also result from excretion processes: large amounts of NH_4^+ -N have been frequently measured in fresh earthworm casts (Syers et al., 1979; Mulongoy and Bedoret, 1989; Lavelle and Martin, 1992), in the latter study probably due to the excretion of ammonium into the gut by endonephridia. In earthworm casts, NH_4^+ -N represents a temporary form of mineral N that is rapidly transformed to NO_3^- by nitrification usually occurring within days (Fig. 5).

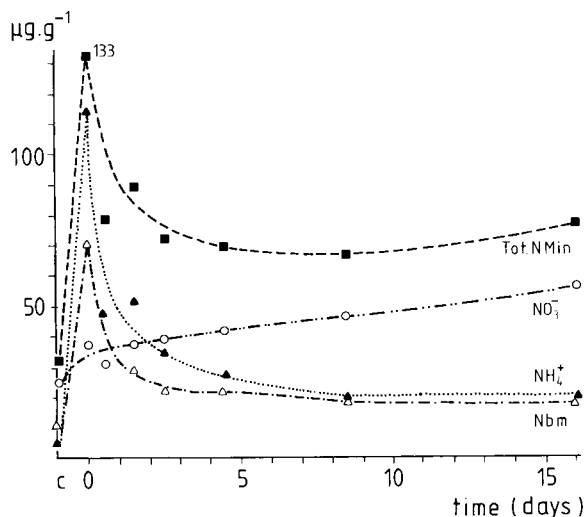


Fig. 5. Changes in mineral-N content and N accumulated in microbial biomass in casts of a tropical earthworm (*Pontoscolex corethrurus*) during the 16 days following deposition. (From Lavelle and Martin, 1993.)

Earthworms may thus increase the short-term availability of mineral N (Spain et al., 1992) and P (Mansell et al., 1981) derived from litter or soil. Similar conclusions have been formulated for soil-feeding termites from a tropical forest by Anderson and Wood (1984) who reported much higher amounts of mineral N and P in termite mounds than in the surrounding soil.

As a result of selective ingestion by animals, the organic matter content of soil fauna excrements may be different from that of the surrounding soil or litter, consequently influencing the chemical properties of the excrements. For example, anecic earthworm feed on a combination of litter and soil, and therefore produce casts with a higher organic matter content and higher cation exchange capacity than the soil (Lal, 1988; Mulongoy and Bedoret, 1989). Moreover, fragmentation of organic particles resulting from digestion processes may increase the availability of organic matter in fresh excrements (S. Martin, 1991), and be responsible for the increase of microbial activity.

The changes of organic matter content may be used to characterize the long-term decomposition processes occurring in soil fauna excrements with high structural stability. Recent laboratory experiments consisting of long-term incubations (S. Martin, 1991) have indicated that the annual decomposition rate was three times lower in the casts of a tropical earthworm than in the surrounding soil; this result suggests that the compact structure of earthworm casts may protect the organic material within the cast from decomposition (Fig. 6). This effect may significantly affect C cycling in the savannas if casts maintain their physical structure for more than 6 months (S. Martin, 1991),

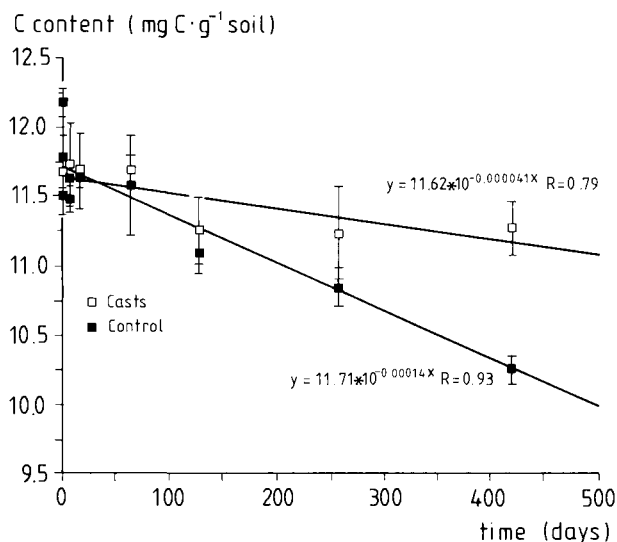


Fig. 6. Evolution over time of C content in 2 mm sieved savanna soil and in casts of a tropical earthworm (*Millsonia anomala*) fed on that soil. Samples were incubated in the laboratory at constant moisture (15%) and constant temperature (28 °C). (From A. Martin, 1991.)

a period of time consistent with field observations (Blanchart et al., 1990). A similar reduction of the rate of decomposition of organic materials in ageing excrements has been reported in enchytraeids (Wolters, 1988) and earthworms from temperate areas (Shaw and Pawluk, 1986a; Morgun, pers. commun., 1991), millipedes (Nicholson et al., 1966) and isopodes (Reyes and Tiedje, 1976; Griffiths et al., 1989).

PHYSICAL PROCESSES IN EXCREMENTS OF SOIL FAUNA

The biological processes in excrements of soil fauna are related to the specific environmental conditions within the excrement. The gut transit which induces addition of water, intense mixing and solubilization, leads to dramatic structural rearrangements of ingested material and to changes in clay/organic matter interactions. The rearrangement of the ingested material may result in a strong compaction within the excrement, influencing microporosity and consequently the water and oxygen availability. A precise description of the physical changes which occur during and after the formation of excrements, is a basic requirement to understand some of the specific biological processes occurring in excrements, because decomposition processes depend strongly on clay/organic matter interactions, aggregation, water and oxygen availability.

Aggregation

The study of the structural stability of soil fauna excrements is important in order to be able to evaluate (1) the potential of soil fauna activity for the formation of soil structure and aggregation patterns, and (2) the physical availability of organic material in the excrements as substrate for soil animals and microbes. E. Blanchart (pers. commun., 1989) observed, for example, that tropical geophagous earthworms never reingest their own casts as long as the casts maintain their physical structure. Stability in itself is not protecting the organic matter, but stable excrements are more resistant to abiotic breakdown, thus preventing the inside of excrements to be exposed to the outer world.

Micromorphological observations of excrements of soil fauna on thin sections by polarizing or electron microscopy (see recent methodological review in Kooistra, 1991) revealed dramatic rearrangements of the soil fabric and litter as a consequence of the digestion and casting processes. Observations on thin sections of fresh and ageing casts of earthworms after selective chemical pretreatment indicated that earthworms may enhance soil aggregate stabilization by reinforcing bonds between clay-polyvalent cations and organic matter and between clay and organic fragments due to the release of neutral sugars and polysaccharides during gut transit (Shaw and Pawluk, 1986b; Shipitalo and Protz, 1989; Barois et al., 1993). Presumably this effect, which could be enhanced by drying–rewetting cycles, is of particular importance for the stabilization of ageing casts and may be similar to the aggregation processes occurring within the rhizosphere (Pojasok and Kay, 1990).

Methodologies developed for testing soil aggregate stability by dry sieving (Van Steenberg et al., 1991) or clay dispersion in water (Shipitalo and Protz, 1989) have recently been used to test the stability of some soil animal excrements. Garnier-Sillam et al. (1988) reported that termite mounds, formed by faecal pellets of soil-feeding species, have a higher structural stability than the surrounding soil. Conversely, Marinissen and Dexter (1990) indicated that although fresh earthworm casts are easily dispersable aggregates, the surface-related stability of casts rapidly increases during ageing, probably due to the growth of superficial fungal hyphae.

Porosity and fluid diffusion

Micromorphological observations have shown that excrements of soil animals may have a very compact structure. Blanchart et al. (1993) reported for example that the bulk density of fresh casts of soil-feeding earthworms may be 1.5 times higher than that of control soil. Also De Vleeschhauwer and Lal (1981) reported a higher bulk density and a lower porosity in earthworm casts than in other soil aggregates. Woodlice excrements may also present a

similar compact aspect, which might prevent the penetration of fungal hyphae, limiting their activity to the surface of the faeces (Hanlon, 1981). The compaction of the excrements might result from irreversible shrinking during drying, a process occurring rapidly in freshly deposited excrements which initially contain large amounts of water (Oades, pers. commun., 1991). Conversely, some studies reported lower density for earthworm casts than for field aggregates (Joschko et al., 1989; Elliott et al., 1990). It is possible that there is a difference between earthworm species in the porosity of the casts they produce; it also may be possible that earthworms produce casts of different bulk density under different conditions.

Recently, the characterization of the pore size distribution in earthworm casts of *Pontoscolex corethrurus* by mercury intrusion indicated that the outer parts of these casts consisted of a very compact structure, the pores mainly very small (mean pore diameter: $10\text{ }\mu\text{m}$), which might reduce water and oxygen movement (Blanchart et al., 1993). The restricted water movement and oxygen supply in earthworm casts might induce anoxic conditions, promoting denitrification (Elliott et al., 1990) and/or preventing organic matter decomposition (S. Martin, 1991).

Direct measurements of nitrous oxide released under oxic or anoxic conditions, revealed that excrements of earthworms are indeed potential sites for denitrification. In laboratory incubations, Svensson et al. (1986) and Elliott et al. (1990) concluded that fresh surface casts of earthworms are microsites

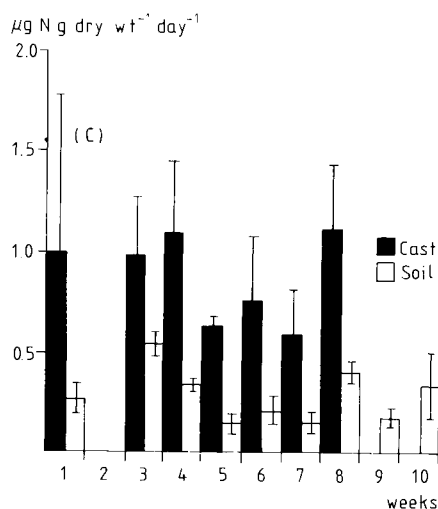


Fig. 7. Production of N_2O ($\mu\text{g N}\cdot\text{g dry wt}^{-1}\text{ day}^{-1}$) from earthworm casts (dark columns) and soils (open columns) incubated with acetylene. Material was collected from October 8 to December 12, 1988 from a drained pasture, supplemented with N fertilizer ($200\text{ kg N ha}^{-1}\text{ yr}^{-1}$). (From Elliott et al., 1991.)

which may produce significant amounts of nitrous oxide and that earthworm activity is likely to affect N cycling in temperate pastures, particularly when large amounts of mineral N are available in the soil (Fig. 7). However, Rapoldt (unpubl. results, 1991) states that anoxic conditions did only occur deeper than about 4 mm in a column of slaked material of casts of *Aporrectodea caliginosa*. This means that under field conditions, where the casts are surrounded by air-filled pores, and do not have a diameter much larger than 5 mm, denitrification would not be expected. This example illustrates the need for studying biological and physical processes interactively.

A relevant description of the physical processes occurring in soil faunal excrements may be useful to better understand some of the biological processes prevailing in these aggregates. For example, the reduction of the decomposition rate of organic matter in excrements, in comparison with surrounding soil or litter, which has been observed for many taxonomic groups, may partly be related to physical processes:

- the close association between clay and organic material, providing a protective coating of organic particles by clay (Blanchart et al., 1993);
- a low oxygen availability within the excrement because of the small pore size (continuously water-filled) either in the outer border of casts (Blanchart et al., 1993) or in the whole cast, due to compaction (Bal, 1973);
- according to Poiseuille's law, movement of water is low in very small pores. This could mean, that also transport of bacteria into the small pores of excrements may be hampered, so that they are unable to reach any organics present in the excrement when large pores are lacking.

These three factors may be responsible for physical protection of organic matter in many excrements of saprophagous soil fauna.

CONCLUDING REMARKS

This review emphasizes the importance of physical processes acting as regulators of biological processes and the need for studies relating morphology, biological processes and physical processes in soil faunal excrements. This knowledge is necessary to evaluate the importance of the saprophagous soil fauna for the functioning (e.g., nutrient cycling, decomposition, humus formation) of the (soil) ecosystem.

Short-term processes in faeces of a variety of soil animals differing in diet and ecology, are well-described: within a large range of animals, fresh casts are characterized by specific microbial communities, a high microbial activity, a high organic matter and nutrient availability and a specific structural arrangement. However, knowledge is still lacking for many long-term processes occurring in different types of excrements. Some excrements are very stable and seem to persist unchanged for a long time, while others disintegrate quickly. It is important to establish, whether physical protection may be the

predominant factor in the regulation of decomposition of organic material in excrements of soil fauna. There are situations where successive reingestions of excrements increases availability of organic material, e.g. by coprophagous species which produce excrements with low structural stability (Gunnarson and Tunlid, 1986). This illustrates the need for future research focusing on the mechanisms which are responsible for an increased or decreased organic matter breakdown in the excrements of a variety of saprophagous soils animals.

A relevant description of processes occurring in soil fauna excrements is a basic requirement to evaluate the contribution of soil animals to soil functioning. Many field studies have provided qualitative information regarding the impact of the soil fauna on soil processes such as fragmentation, mineralization and incorporation of litter into the soil, soil layers translocation, macroporosity distribution and aggregate formation. However, only few quantitative data are currently available. Such quantitative information may be obtained by extrapolating results of laboratory studies dealing with soil animals to the scale of ecosystem. To do this, some background information is needed: (i) a proper description of the ingested material, (ii) a realistic estimation of ingestion rates, and (iii) a description of factors determining ingestion rate. Combining this data with a simulation model of population dynamics, S. Martin (1991) extrapolated laboratory data of mineral N production in tropical earthworm casts to the scale of the ecosystem and calculated that earthworm populations may contribute $\pm 15\%$ of soil N mineralization in a tropical savanna. The significant contribution of soil fauna to soil processes may partly explain the rapid degradation of soil fertility in cultivated soils, frequently observed in the humid tropics, as a result of soil fauna elimination (Lavelle and Pashanasi, 1989).

Future studies of the long term effects of soil fauna on decomposition should consist of combinations of carefully chosen methods on morphology, microbial activity and physical characteristics.

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Concepts and methods for studying interactions of roots and soil structure ^α

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ABSTRACT

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The central concept in present-day root ecological studies in agriculture is that reduced or improved root development may decrease or increase the efficiency of using water and nutrients and thus aggravate or reduce negative environmental impacts of agriculture. The normal root development of many agricultural crops is such that chemical fertility of the soil has to be so high that residues of NO_3 and P in the soil remain at harvest which are close to or higher than those acceptable to meet environmental standards on quality of ground- and surface water. Poor soil structures, with a large fraction of the soil at a relatively large distance to the nearest macropore or crack, may affect root penetration and functioning, and thus lead to even higher residues of macronutrients at harvest. If roots are not able to penetrate the matrix of the aggregates a clustered root distribution in the cracks is the result. Negative effects on nutrient and water uptake efficiency of non-regular root distribution and incomplete root–soil contact can now be estimated from the Root Position Effectivity Ratio, R_{per} . Aeration of roots depends on the (maximum) distance between root tissue and the nearest “breathing section”, where part of that root is in contact with air-filled macropores or cracks, on the air-filled porosity of the root and on protection against leakage of oxygen out of the root.

Methods are discussed for measuring: distance in pixel images, root distribution pattern on maps, synlocation of roots and other map features, root–soil contact, root position effectivity ratio, air-filled root porosity and continuity of air channels, and dynamics of root growth and decay.

1. INTRODUCTION

1.1. Roots as soil biota

Roots are a special category of “soil biota”, as they are part of an organism which lives in two very different environments, the (upper layer of the) soil

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and the (lower part of the) atmosphere. Three levels of complexity in root ecological studies can be distinguished (Van Noordwijk, 1993a): (A) studies of single roots interacting with their environment; (B) studies of root systems in a homogeneous environment, including root–shoot interactions; and (C) studies of root systems in a heterogeneous root environment, including root–shoot interactions. Penetration of dense layers of soil has often been studied at the single root level. When studied from a whole plant perspective (level B), negative effects of soil compaction can often be compensated by improved supply of water and nutrients (Schuurman, 1971). When studied in a heterogeneous environment (level C), penetration of dense soil layers may depend on conditions around other roots or other parts of the same root. Figure 1 shows that penetration of oat roots into a “very dense” soil layer (bulk density 1.52 g/cm^3) was better when the topsoil was “dense”, than when it was “loose” (bulk densities 1.38 and 1.24 g/cm^3 , respectively; Schuurman

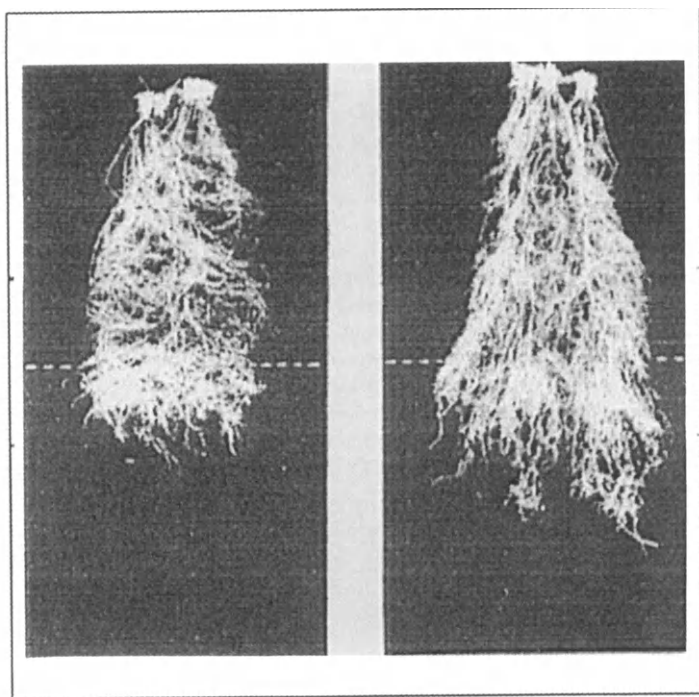


Fig. 1. Penetration of oat roots in a “very dense” sandy subsoil (bulk density 1.52 g cm^{-3}) from a “loose” (b.d. 1.24 g cm^{-3} ; left) and “dense” (b.d. 1.38 g cm^{-3} ; right) topsoil in a tube experiment of Schuurman and De Boer (1974).

and De Boer, 1974). A method for studying root response to local variation in soil density in the field was described by Hairiah et al. (1991).

In the past, a “morphogenetic equilibrium” between roots and shoots was the central concept in root ecology. Any reduction in root growth, e.g., due to a poor soil structure, was assumed to reduce shoot growth. During the last three decades the interactions and feedback controls between shoot and root are interpreted as a “functional equilibrium”. Increased supply of water and nutrients can (largely) compensate for reduced root development in many situations (Van Noordwijk and De Willigen, 1987). The central concept now is that reduced root development may affect the efficiency of the use of water and nutrients and may enhance negative environmental impacts of agriculture (Van Noordwijk and De Willigen, 1991).

Figure 2 summarizes a possible causative chain (model) for effects of poor soil structure on environmental pollution. A causative chain can be represented as a series of links between “Patterns” and “Processes”. Patterns act as initial and boundary conditions for processes; processes lead to new patterns, at a higher level of complexity. Patterns are observed more easily than processes; processes can be better generalized and have a more universal value. A relation between soil structure (pattern) and environmental pollution (process) could be based on:

(a) Poor soil structure negatively affects root development leading to a smaller or less effective root system, more susceptible to aeration stress and root death after heavy rainfall.

(b) A smaller root system is less effective in the process of nutrient and water uptake and will leave a larger nutrient residue at harvest time; as the lower efficiency is predictable, the farmer will increase chemical soil fertility to compensate for yield effects, but increasing the residue as well.

(c) Large nutrient residues at harvest time will lead to high nutrient concentrations in the leachate during winter rains, polluting the environment.

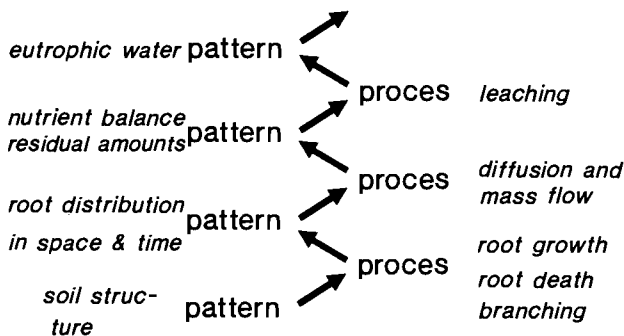


Fig. 2. Interrelationships between patterns and processes linking soil structure, root growth and functioning and environmental effects of agriculture.

1.2. Uptake efficiency

Agronomic nutrient or water use efficiency on the field scale depends on variability in supply and demand between individual plants on a field which is managed as if it were a homogeneous unit (Van Noordwijk and Wadman, 1992) and on three components of efficiency within the area exploited by a single plant:

- application efficiency: available supply per unit input; the available fraction is defined with respect to the time period relevant for the crop under consideration;
- uptake efficiency: uptake per unit available nutrient or water;
- utilization efficiency: crop production per unit uptake.

The uptake efficiency depends on the root system and will be the main focus of attention here. As analyzed by De Willigen and Van Noordwijk (1987), nutrient or water uptake efficiency depends on the required uptake rate per unit root (which is related to shoot/root ratio and growth rate of the plant), on the degree of synlocation of roots and resources (depending on resource mobility and soil water content) and on the degree of synchrony of resource supply and demand (depending on resource buffer capacity).

Five levels of modelling nutrient and water uptake by crops can be distinguished (Van Noordwijk and Van der Geijn, 1993):

(O) models without roots, calibrated on nutrient uptake efficiency (NUPE) and water uptake efficiency (WUPE);

(A) models evaluating uptake efficiency of a given root system characterized by its root length density, degree of root–soil contact and root distribution pattern;

(B) as (A), but using curve fits on root dynamics per depth layer or radial zone around the plant, to describe root development and root decay during the growing season;

(C) as (A), but including a “functional equilibrium” type of shoot–root interaction during crop development;

(D) as (C) but including “local response” to heterogeneity of resources and stress factors.

For direct applications, model level O may be desirable for its simplicity; it may be sufficient, provided that models are adequately calibrated and are not used for extrapolation to new situations. Complexity increases stepwise for models A...D. Model level A is needed for more complex models.

An important reference point for model levels A...D is formed by the potential uptake rate of a single root in the centre of a cylinder of homogeneous soil, in full contact with the soil. The radius of this soil cylinder is derived from the root length density, assuming a regular root distribution and parallel roots. As diffusive transport is based on concentration gradients it can not supply all available nutrients to the root surface in a restricted period of time

and a certain residue will remain in the soil, especially in situations where crop demand determines uptake and hence crop growth is not restricted by the supply of the resource studied. If crop demand and nutrient supply are completely predictable for the farmer, this residue can—without affecting uptake rates—be reduced to a minimum value C_m , such that the potential uptake rate A_p just equals the required rate A_r at the end of the uptake period (De Willigen and Van Noordwijk, 1991):

$$C_m = C_{\text{lim}} + \frac{A_r G(\rho, \nu)}{\pi H D_\theta L_{rv} (\rho^2 - 1)} \approx C_{\text{lim}} + \frac{A_r D_r^2 G(\rho, \nu)}{4 H D_\theta} \quad (1)$$

where:

C_m = minimum average concentration in soil solution at end of uptake period (mg cm^{-3}),

C_{lim} = concentration at root surface area required for physiological uptake process (mg cm^{-3}),

A_r = required uptake rate per unit soil surface ($\text{mg cm}^{-2} \text{d}^{-1}$)

H = depth of soil layer (cm),

D_θ = effective diffusion coefficient for solutes, ($\text{cm}^2 \text{d}^{-1}$), dependent on soil water content θ ,

L_{rv} = root length per unit volume of soil (root length density) (cm cm^{-3}),

R_1 = radius of soil cylinder = $(\pi L_{rv})^{-0.5}$,

ρ = dimensionless cylinder size = $2 R_1 / D_r = 2 (\pi L_{rv})^{-0.5} / D_r$,

D_r = root diameter (cm),

ν = dimensionless flux of water.

$G(\rho, \nu)$ is the dimensionless function of root length density and transpiration rate:

$$G(\rho, \nu) = \frac{1}{2(\nu+1)} \left(\frac{1-\rho^2}{2} + \frac{\rho^2(\rho^{2\nu}-1)}{2\nu} + \frac{\rho^2(\rho^{2\nu}-1)(\nu+1)}{2\nu(\rho^{2\nu+2}-1)} + \frac{(1-\rho^{2\nu+4})(\nu+1)}{(2\nu+4)(\rho^{2\nu+2}-1)} \right) \quad (2)$$

The right-hand side of eq. (1) gives an approximation which is good, unless root length density or root diameter is very high. For a situation without mass flow ($\nu=0$) $G(\rho)$ replaces $G(\rho, \nu)$:

$$G(\rho) = \frac{1}{2} \left(\frac{1-3\rho^2}{4} - \frac{\rho^4 \ln \rho}{\rho^2-1} \right) \quad (3)$$

This relatively simple cylindrical model can be used to deal with situations with a more complex geometry. A non-regular root distribution, even in combination with incomplete root-soil contact, in a layer with given nutrient concentration and soil water content can be represented by a series of cylinders, rather than by a single one. The potential uptake rate for such a situation is

obtained by the weighted average of the uptake for each cylinder. In the approximate version of eq. (1), total uptake of homogeneously distributed resources will be proportional to a sum of G -functions in stead of a single function $G(\rho, \nu)$. An "effective" root length density L_{rv*} can be found for which the G function equals this sum of G -functions (see section 3.5). The root position effectivity ratio, R_{per} , is defined such that $R_{per} = L_{rv*}/L_{rv}$, where L_{rv*} is the effective and L_{rv} the real root length density. L_{rv*} can be used for calculations of uptake rates, because the G -function appears in dynamic uptake models for all nutrients and water. By definition R_{per} will be between 0 and 1, as non-regular root distributions are less efficient in utilizing homogeneously distributed resources. For clustered resources the sum of the products of C_i and $G(\rho, \nu)_i$ should be considered and an "effective" root length density at the average concentration can be above obtained. In this situation R_{per} can be above 1.0 (synlocation, section 3.3). A method to compute R_{per} for observed root distributions will be presented in section 3.5.

1.3. Root development needed to meet environmental standards

The hypothesis that a better root development may help to reduce pollution needs quantification: how many roots are needed to reduce pollution to acceptable limits? Environmental standards are now proposed in The Netherlands and will (hopefully) soon be effectuated to restrict residues of macronutrients at harvest time to such a level that water leaching through arable soils has an acceptable quality. For nitrate the standard is based on an EC standard for drinking water of 50 mg/l (or 11.3 mg/l of N). Given a rainfall excess in winter of about 300 mm (3×10^6 l/ha) a maximum $\text{NO}_3\text{-N}$ content in the rooted zone of 34 kg/ha can be derived. When allowance is made for some denitrification, a maximum residue of 45 kg/ha is now formulated as a future policy target and 70 kg/ha as immediately applicable standard (Neeteson, 1993). Because part of this residue will be due to spatial variability within a field and to mineralization after crop N uptake has diminished, the $\text{NO}_3\text{-N}$ residue within the root zone of a reference plant should be less than 20 kg/ha (Van Noordwijk and Wadman, 1992). The root length density required to achieve this target depends according to eq. (1) on daily crop demand for N (A_r) and soil water content (θ); as a first estimate effective root length densities in all zones containing NO_3 above 0.3 cm/cm^3 will be sufficient. As NO_3 will not only occur in the plough layer, subsoil root development may be critical for meeting environmental standards for NO_3 . For K, a groundwater standard of 12 mg/l can be met if the residue of available K is less than 360 kg/ha at harvest, when we assume linear adsorption of K and an apparent adsorption constant of 10 ml/cm^3 (De Willigen and Van Noordwijk, 1987). Effective root length densities in the K-containing layers of at least 1 cm/cm^3 are needed to achieve this target. For P a maximum concentration in surface

water of 0.15 mg/l can be met if leachates are equilibrated with soil with a P_w value of 12 to 17 mg P_2O_5 per l depending on soil type (Van Noordwijk et al., 1990). Of the main arable crops only wheat—with a root length density in the plough layer of about 5 cm/cm³—will grow unrestrictedly at such a level of available P. For other crops, with a lower root length density, a higher available P supply is maintained in the plough layer, so a potential risk of pollution exists. For NO_3 in subsoil and P in the plough layer we may conclude that the actual root development of arable crops is critical if environmental standards are to be met without inducing nutrient stress to the crop. Reductions in root development and in effectivity of the root system for uptake, which may be the results of a poor soil structure, can therefore be unacceptable.

1.4. "Good" soil structure

The concept of a "good" or "poor" soil structure should be defined before we can investigate its relations with root development. By putting a spade in the ground and observing the soil on a broken surface, large differences in soil structure can be noted (Preuschen, 1983). By comparison with photographs of reference structures, a rating from 1 (very bad) to 10 (excellent) was developed in The Netherlands and was used extensively to evaluate effects of soil tillage and other soil management practices (Boekel, 1982). Although valuable as such, a better quantification is needed to link soil structure to other processes, such as transport of water, nutrients and gasses and root development. Boone (1988) developed a functional interpretation scheme for soil compaction, based on the width of the interval in soil water content between "too wet" for aeration and "too dry" for root growth. Lipiec et al. (1991) used a "degree of compactness" by taking the ratio of the actual bulk density and the maximum bulk density obtained by a static pressure of 200 kPa. For both a silty loam and a loamy sand a value of 90% appeared to be critical for spring barley. If maximum penetration resistance is 3 MPa and 10% air-filled porosity of the soil is needed for aeration, water tension may range between -800 and -10 kPa on the silty loam and between -200 and -10 kPa on the loamy sand, at 90% compactness. Critical values on both sides of the range depend on crop species and other growth conditions.

Apart from the problem of finding the relevant parameters, the applicability of such schemes to aggregated soils may be questioned on conceptual grounds, as important aspects of "structure" are not represented adequately by bulk density. Modern soil micromorphological techniques can be used to quantify void continuity and to relate transport properties (processes) to observable patterns (Kooistra, 1990). Ismail (1975) developed a classification scheme for void pattern distribution by quantitative image analysis techniques on thin sections, based on area and diameter of voids. Ringrose-Voase

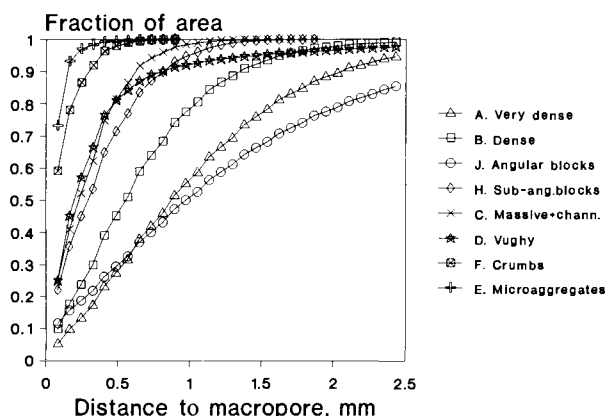


Fig. 3. Cumulative frequency distribution of nearest neighbour distances for points in the soil matrix to the nearest macropore or gap for 8 different soil structures, based on digitized images of polished thin section faces of Ringrose-Voase (1987).

(1987) gave a similar scheme for quantitative description of soil macrostructure by image analysis of polished thin section faces. In soils with fine aggregates, all area is close to the nearest macropore or gap; in soils with large blocks a larger fraction of the area on a soil surface will have a relatively large distance to the nearest macropore or gap. In Fig. 3 the frequency distribution of soil-macropore distances is quantified for eight soil structures identified by Ringrose-Voase (1987). If small, round pores—which may be actually filled by roots—would be neglected, distances would be much larger. In the Dutch visual rating system, these structures range from poor (3) to very good (8) (Boekel, pers. commun.). A method used to measure distances in such images is presented in section 3.1. Such a distance classification can be used for modelling oxygen diffusion in soil (Rappoldt, 1991), as an alternative to actual separation of aggregate sizes by sieving. The transition from two-dimensional observations to three-dimensional reality needs some special attention. Ringrose-Voase (1987) discussed this transition on the basis of a shape classification of macropores and cracks.

2. INTERACTIONS BETWEEN ROOTS AND SOIL STRUCTURE

2.1. *Effects of roots on soil structure*

By extracting water roots may enhance drying-wetting cycles in the soil and thus modify soil structure. Roots may even influence zones of the soil without or at least before penetrating them. As penetration resistance increases when a soil dries out, water uptake may reduce or prevent subsequent root penetra-

tion of that or a neighbouring layer of the soil in soils with sufficient capillary rise (Bengough, pers. commun.).

Roots may enhance or reduce inter-aggregate binding through exudates, decaying tissues and root-associated fungal networks. The long-term effect of the presence of roots in a layer will normally be to enhance aggregate stability, but reductions of aggregate stability by root exudates are possible in the short term. Physical movements of the root tip (see below) might add to the loosening effect. Loosening of aggregates would—similar to the effects of mechanical disturbance or soil tillage (Nordmeyer and Richter, 1985)—increase the accessibility of soil organic matter to bacteria and/or accessibility of bacteria to the next steps in the food chain and thus stimulate N-mineralization. Structural deaggregation by root activities and enhanced N-mineralization may provide an alternative explanation for some of the evidence for N_2 fixation in the rhizosphere. Griffiths and Robinson (1992) showed that the (observed) stimulatory effects of plants on the net mineralization of soil organic nitrogen can not be adequately explained by the C in exudates as energy source for microbial activity; but the presence of roots might improve the availability of other energy sources. An exudate front diffusing ahead of a relatively slow growing root may induce a microbial (or soil structural) response in the soil before the root tip has arrived (Darrah, 1991). McCully (1987) described that young maize roots are closely associated with the soil matrix and appear as “sheathed” roots when extracted from soil, while older roots are “bare” and have less contact with the soil. It seems that the close association between a root and its soil sheath can not be obtained without a disruption of existing aggregates and a subsequent re-stabilization of soil material around the root. Jaillard (1987) observed a reorientation of clay in the near rhizosphere by micromorphological techniques. We formulate a new hypothesis here that effects of exudates and/or root movements on destabilizing soil structure may be responsible for enhanced N-mineralization and for re-orientation of soil aggregates.

2.2. Effects of soil structure on roots

Soil structure may influence root development and thus resource accessibility for the plant as a whole. Four aspects will be considered here: (1) effects on root length density; (2) effects on root distribution pattern (section 3.2); (3) effects on root–soil contact (section 3.4); and (4) effects on aeration (section 3.6) and hence on dynamics of growth and decay of the root (section 3.7).

Much attention has been given to the ability of roots to penetrate a homogeneous compacted layer of soil. In the last decade the response of roots to aggregate surfaces, encountered after passing through a gap or void, has also been studied (Hewitt and Dexter, 1984). Much less attention was given to

spiral movements of the root tip under such circumstances (Spurny, 1966), described as “circumnutation” in old plant physiological literature (Darwin, 1880). Barlow et al. (1991) studied geotropic response of roots at the cellular level and found evidence for spiralling movements as well. Circumnutation of root tips may be important as it allows a root tip—with the possible use of mucigel as a glue—to grow along aggregate faces and establish partial root–soil contact when a root enters a gap or macropore (Kooistra et al., 1992). For many agricultural crops the rate of elongation may decrease to about one half by rather small mechanical stresses (20 to 50 kPa) (Lucas, 1987). If such retardation would happen on one side of the root only in roots growing on an aggregate surface in a gap or macropore, it would form an efficient mechanism to follow this surface, even where it bends away.

Roots penetrating the soil matrix will have complete contact with the soil matrix and micropores, which will be waterfilled when the soil is at field capacity. Complete root–soil contact facilitates uptake of water and nutrients, but aeration of the root may be difficult. A certain degree of air-filled porosity of the cortex is necessary to allow oxygen to diffuse to all cells of the root in this situation. The porosity required depends on the distance to the nearest “breathing section” on the root, where gas exchange with a macropore is possible. Cellular structure outside the cortex (epi- and exodermis) may influence entry of oxygen in the “breathing section”, but also loss of oxygen out of the root tissue in the remaining part of the root. Models of longitudinal transport of oxygen in roots (De Willigen and Van Noordwijk, 1989) so far assume this radial transport resistance to be uniform, but critical measurements to support or refute this assumption are lacking. While a root tip is penetrating into an aggregate, aeration is problematic, because cortical air channels do not extend to the tip where metabolic activity is highest and oxygen has to diffuse across almost the complete diameter of an aggregate (Fig. 4). After

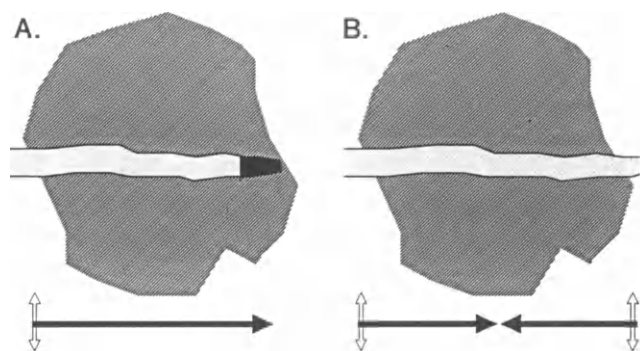


Fig. 4. While a root is penetrating an aggregate the internal oxygen transport must bridge almost the complete diameter of the aggregate (A); once the root tip has emerged into a new macropore or crack where oxygen is available, the transport distance is reduced to the aggregate radius (B).

the root has entered a new macropore only the radius of the aggregate has to be bridged, from two sides. Additionally, oxygen demand per cm of root length for maintenance and uptake will be less than that required for root growth (Lambers, 1987). Once formed, a root may thus survive a certain reduction in oxygen supply when the soil becomes wetter. When the soil becomes waterlogged, however, the oxygen stored in air-filled pores may be depleted within a day (Boone and Veen, 1992). Physiological mechanisms to survive temporary aeration stress may be essential (Drew, 1990), unless internal oxygen transport from aboveground tissues is possible. Plant species differ considerably in air-filled root porosity and also in the ability to increase such porosity when aeration is insufficient. Cortical air spaces are normally continuous within a single root axis, but barriers may exist between main axes and branch roots. A method to study air-filled porosity and continuity of air spaces is described in section 3.6.

Apart from the two classical descriptions of root methods by Schuurman and Goedewaagen (1971) and Böhm (1979), several recent reviews are available on methods to quantify root development and functioning in the field: Anderson and Ingram (1989), Caldwell and Virginia (1991), Jaillard (1987), Mackie-Dawson and Atkinson (1991), Taylor et al. (1991), Van Noordwijk (1987). We will concentrate here on some new methods to study the interaction of roots and soil structure, relevant to the concepts discussed above.

3. METHODS

3.1. Distance measurements in pixel images

As diffusion depends on gradients in concentration, model presentations should try to conserve the frequency distribution of distances between “source” and “sink” in the transport process (Van Noordwijk, 1987; Rappoldt, 1991; Tardieu, 1991). The traditional way of measuring “nearest neighbour” distances (Diggle, 1983) is to take a large number of randomly located points, measure the (Euclidean) distance to all objects of interest and choose the smallest distance. The process can be speeded up by using algorithms to restrict the number of potential neighbours for which distances have to be measured (Diggle, 1983). An alternative method to measure the frequency distribution of nearest neighbour distances is based on the fact that a circular disk of radius r contains all points with a distance to the circle centre less than or equal to r . By drawing circles of radius r around all objects of interest and measuring the area within the union of all these circles, the area fraction within distance r can be measured. By repeating this procedure for increasing values of r , until the whole area of the sample is included, the complete frequency distribution of nearest neighbour distances can be measured.

Computer image analysis is normally based on digitized arrays of square pixels. Although real circles cannot be presented, they can be closely approximated; measurements of area within the union of circles around objects of interest, is easily done by counting the number of pixels within that union. Several image analysis programs contain “distance transforms”, which classify pixels by the radius of an approximated circle needed to reach them from the nearest object of interest. If such distance transforms are not available, and when the presence of barriers complicates distance measurements, a sequence of dilation or expansion steps can be used. Two basic forms for expansion exist, a 4-connective one where each pixel has four and a 8-connective one where it has eight neighbours. Alternating these two forms in a 2:1 ratio, in a 4–8–4 sequence, an octagon (Fig. 5A) is created which has a surface area close to that of a circle with a radius n , for each step in the sequence. By

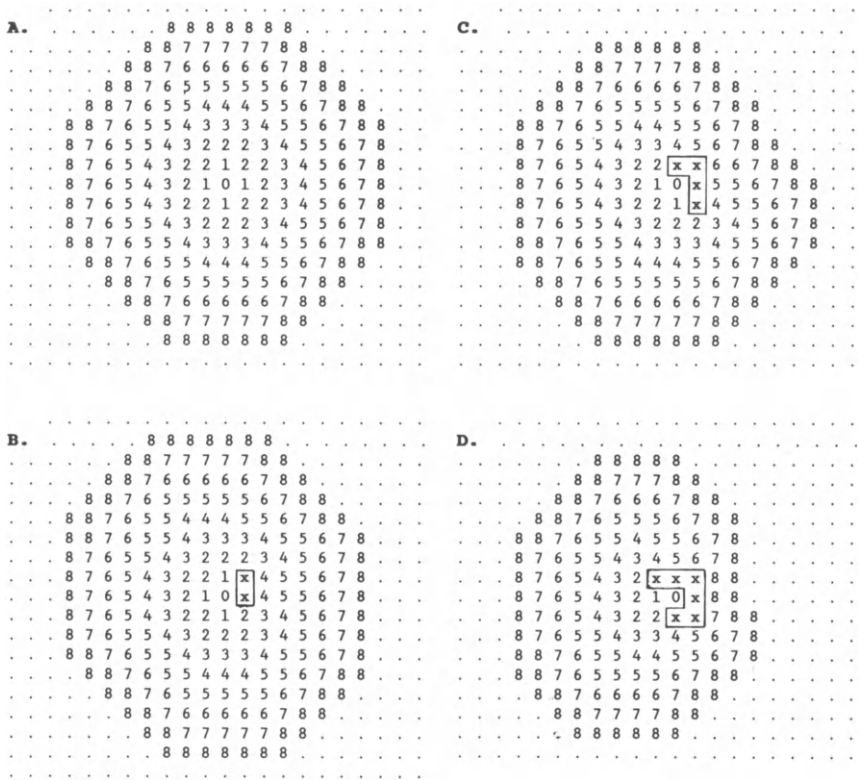


Fig. 5. Classification of area according to distance to the nearest root for the first 8 expansion steps around a root located at 0, for a root with complete (A) or incomplete root–soil contact (RSC) (B–D). (B)=75, (C)=50 and (D)=25%. Incomplete root–soil contact is represented by a number of x -pixels not included in the expansion. Distances were derived from a sequence of 4/8/4-connective steps (Kooistra et al., 1992), that means step 2, 5 and 8 are 8-connective.

skipping one 4-connective step after every 29 steps, the circle approximation can be improved (Kooistra et al., 1992). Figure 3 was derived using such an algorithm. Barriers can be introduced by—each step—subtracting the image of the barriers from the expanded image before measuring the area.

3.2. Root distribution pattern on soil maps

Root distribution can be observed on horizontally and vertically oriented planes in the soil. The relation between root length density, L_{rv} , and the number of root intersections per unit surface area, N , can be described as: $L_{rv} = C_0 X N$, where C_0 is a (theoretical) calibration factor depending on root orientation and X an additional empirical calibration factor due to sampling errors. If roots are randomly oriented C_0 is 2.0. For non-random orientation of roots C_0 is close to 2.0 if N is the average point density in three mutually perpendicular planes of observation in the middle of the soil volume, but will vary between 1.0 and infinity if N is based on only one plane of observation (Van Noordwijk, 1987). For plane(s) of observation outside the volume of soil, a further correction factor is needed to estimate the number of intersections in the middle of the volume of soil; this correction is usually neglected but may be relevant for large volumes of soil or in situations with a pronounced trend in root length density. The empirical calibration factor X is probably due to sampling errors. When calibrating washed root length density against point density on thin sections Van Noordwijk et al. (1992) found X to be 1.0. If point densities are based on the core break method, an empirical calibration factor X of about 3.0 is not uncommon (Drew and Saker, 1980; own unpublished results). When root observations are based on profile walls similar values for X may be found, depending on the method used for preparing the profile walls. Such high calibration factors probably indicate that a major part of the finest branch roots are not recognized and cast doubt on the validity of this method of observing root distribution.

Several methods exist for preparing a profile walls for observation (Böhm, 1979). Removing soil with a brush, fork or pin, spraying the soil with a hand-sprayer, covering the soil overnight with cloth soaked in sodiumpyrophosphate to disperse clay or blowing soil away with an air current may all help, under certain conditions, to improve visibility of roots. A drawback of such methods, however, is that the plane of observation becomes less clearly defined. Certain observation procedures actually record root length in an exposed volume of soil. For studying the relation of roots with soil structure observations in a single plane should be preferred. Spraying the soil with water sometimes leads to smearing of the soil and roots may actually be covered by soil. In our laboratory we currently only smooth profile walls with a sharp blade and do not remove soil otherwise. Further development of techniques based on autofluorescence of roots and/or translocation of fluorescent dyes

applied aboveground might improve observations in future. Work on fluorescent dyes in our laboratory has not been successful so far.

Root patterns differ from regularity for two types of reasons: an inherent pattern, because branch roots originate from main axes and are originally close to them, and an imposed pattern, because soil characteristics such as structure restrict or stimulate root development locally. The first aspect is reflected in a model of the three-dimensional architecture of a maize root system (Pages et al., 1989). This model, and that of Diggle (1988) is based on branching rules as observed on washed root systems grown in soils without structural obstacles. Although at first sight root systems may appear to have a fractal nature, on more detailed analysis branching rules and root orientation change with branching order. The model of Pages et al. (1989) can generate root maps and numerical values of root coordinates for any plane of observation. Analysis of patterns on such maps, which reflect the inherent branching pattern, can be compared with those observed in real soils. Differences in pattern may then be attributed to soil characteristics, such as soil structure.

3.3. Synlocation of roots and resources

Non-regular root distribution in structured soils usually means that nutrients, water and/or oxygen are also non-homogeneously distributed. A measure of spatial correlation between roots and resources (synlocation) is needed to quantify these effects.

Staricka et al. (1991) measured the frequency distribution of residue concentrations per unit soil volume for different soil tillage systems. Local concentrations of several times the average concentration occur on a cm^3 scale with each type of tillage implement. As such residue concentrations can be observed macroscopically, they can be included in root maps. Synlocation of roots and residue can be quantified by measuring the area of, and the number of dots coinciding with, the residue and a series of expanded images (see section 3.1). Point density is thus estimated as a function of distance to the residue and can be analyzed statistically (Van Noordwijk et al., 1993a). Any object which can be observed macroscopically can be included in the map and analyzed similarly.

3.4. Root-soil contact

Roots penetrating the soil matrix will initially have complete contact with the soil matrix plus micropores. Roots growing along aggregate surfaces or in macropores with a diameter larger than that of the root, will have incomplete contact with the soil. Root-soil contact can be directly observed on thin sections of the soil, provided that sample preparation, and especially the dehydration steps involved, did not lead to shrinkage of the root. Tests of the pro-

cedure for a pot experiment were given by Van Noordwijk et al. (1992), results for this pot experiment by Kooistra et al. (1992) and for a field trial by Van Noordwijk et al. (1993b). Jaillard (1987) combined a micromorphological technique with detailed chemical analysis of the rhizosphere.

3.5. Root position effectivity ratio, R_{per}

De Willigen and Van Noordwijk (1987) studied the effects of two types of complications in the geometry of the root-soil system: irregular distribution of roots and incomplete root-soil contact. The solutions given for both aspects separately left a question about combined effects. As both aspects are based on shifts in the transport distances involved, it may be possible to approach them in a similar way and thus include combined effects.

An analytical solution (Veen et al., 1992) showed that the nutrient residue was approximately proportional to the degree of root-soil contact. This solution was based on a sink in the centre of a circle. In a regular distribution of roots, a circle is a reasonable approximation, as it closely resembles the hexagons of a "watershed" division in which all transport is supposed to go in the direction of the nearest sink. We may expect, however, that incomplete root-soil contact will affect the watershed division, and that the root will be eccentric in its water-shed area (Fig. 5). In analyzing the effects of non-regular distribution De Willigen and Van Noordwijk (1987) found that an eccentric position in a soil cylinder of a root with complete root-soil contact reduces the uptake potential. For a root with incomplete root-soil contact, however, an eccentric position in the watershed area, with less soil on the "back" side where no root-soil contact exists, may improve the uptake potential. The increase in nutrient residue by incomplete root-soil contact may be less if the water-shed division is based on actual nearest neighbour distances. With the algorithm described in section 3.1 the frequency distribution of soil-to-root distances can be derived in the presence of air-filled barriers around a root, allowing only partial root-soil contact. Figure 5 shows the first eight steps of such an expansion for a root-soil contact of 100, 75, 50 and 25%, respectively. With decreasing root-soil contact, the root becomes progressively more eccentric in its watershed area, and the analytical solution by De Willigen and Van Noordwijk (1987) may overestimate the effect of incomplete contact.

For the derivation of R_{per} , the frequency distribution of soil-to-root distances should be determined (see Appendix; Van Noordwijk, 1992b). The next step is the construction of a set of circle annuli which conserve the frequency distribution of distances for an average root and to re-interpret this set of circle annuli as a set of circle sectors (Fig. 6; Appendix, points 4 and 5). Rappoldt (1991) described an algebraic solution for deriving sector frequencies from the frequency distribution of distances, if all distance incre-

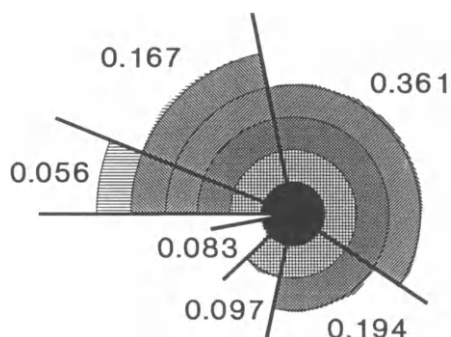


Fig. 6. Annulus fraction presentation of the frequency distribution of distances between (randomly chosen points in) the soil and the nearest root surface, for an average root in a realistic root distribution pattern (each type of shading indicates a different class of distances). Division of this shape into circle sectors allows application of cylindrical uptake models (Appendix; Van Noordwijk, 1993b).

ments (including the first) are equal. If the first increment is half as large, as used for an expansion in a pixel image, a modified algebraic solution is needed, or the iterative procedure used here. All sector frequencies are positive (or zero) when distances are measured on a map without barriers or interruptions. If gaps around the root are included in the distance measurements, some of the sector frequencies are negative.

For each of the sectors the cylindrical model can be applied to predict uptake. For the root system as a whole, the potential uptake rate can be predicted from the weighted sum of the uptake from each cylinder class, subtracting results for any negative frequencies. This approach assumes the average concentration in the soil solution, as well as soil water content to be constant. A partly unresolved question is to which extent and how rapidly physiological interactions occur between uptake rates of roots, if some roots can take up more than others. If supply is limiting uptake (the weighted sum is less than current demand), no such interaction is to be expected and by adding the potential uptake rate for each sector we obtain the potential uptake rate for the root system. Within a single soil layer with a uniform soil water content and average concentration in soil solution, for each root distribution with root length density L_{rv} a uniformly distributed, equivalent root length density L_{rv*} can be found which would leave the same residue in the soil (Appendix, step 6 and 7). R_{per} is now defined as L_{rv*}/L_r (Van Noordwijk, 1993b).

Most values of R_{per} observed so far on root maps from different soil structures are in the range 0.6–0.9. Figure 7 shows R_{per} for root distributions simulated by a layered Poisson process (Diggle, 1983), as defined by three parameters: N (number of dots per 520×520 pixel frame), Relint (average number of roots per group; intensity of the clustering) and Reldis (maximum

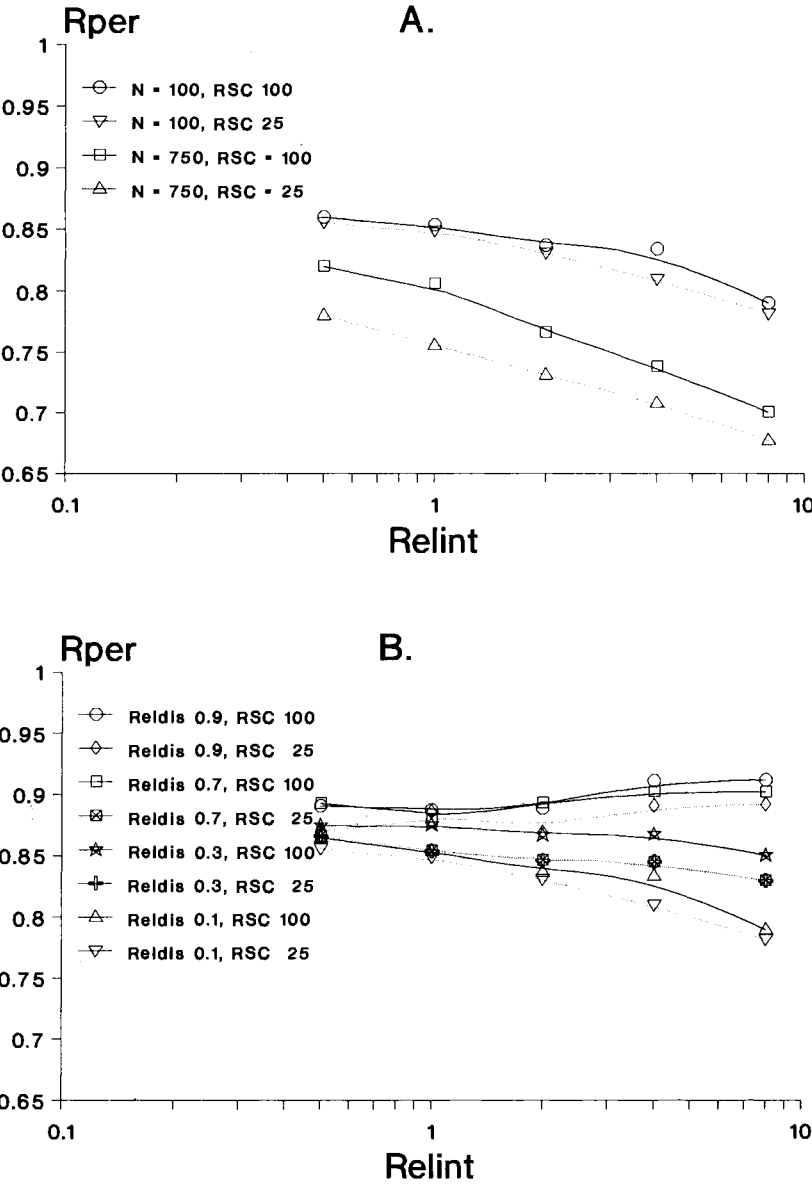


Fig. 7. Root position effectivity ratio, R_{per} , for root maps made in a layered Poisson process, with on average Relint branch roots per cluster and with distances to the main axes of Reldis times the average distance between neighbouring main roots; thick lines for complete, normal lines for 25% root soil contact. (A) Results for a different number of roots per field of view (520×520 pixels), with Reldis=0.1. (B) Results for various values of Reldis for $N=100$.

TABLE 1

Root densities and root position effectivity ratio (R_{per}) for horizontally oriented thin-section samples from three depths at two fields (12B and 16A) of the Lovinkhoeve soil ecology experiment, June 1990 under winter wheat (Van Noordwijk et al., 1993b)

Depth (m)	N/cm^2		$R_{\text{per}}(100)$		$R_{\text{per}}(25)$	
	12B	16A	12B	16A	12B	16A
0.15	4.90	4.25	0.79	0.80	0.73	0.75
0.25	2.85	1.86	0.82	0.82	0.78	0.77
0.40	0.81	0.28	0.88	0.89	0.85	0.87

$R_{\text{per}}(100)$ and $R_{\text{per}}(25)$ were calculated assuming complete or 25% root-soil contact, respectively, for all roots, with random orientation of the root-soil contact zone.

distance between branch roots and main axis, relative to the average distance between neighbouring main roots; grain size of the clusters). For a truly random pattern R_{per} values are 0.85–0.90 for realistic values of N . For clustered patterns R_{per} values in the range 0.7–0.85 are found, depending on N and Relint (Fig. 7A). If Reldis is above 0.5 an increase in Relint has little effect on R_{per} (Fig. 7B).

Table 1 gives some results of R_{per} measurements on the root pattern observed on thin sections of the Lovinkhoeve soil ecology project. Observations were made on the thin sections described by Van Noordwijk et al. (1993b) to avoid sampling errors involved in larger profile wall maps. The R_{per} values found (assuming complete root-soil contact) were in the range 0.8–0.9, close to that expected for random patterns. In Table 1 and Fig. 7 results are included for 25% root-soil contact, as well. The effect of partial root-soil contact on R_{per} are smaller than expected and further checks of the method are needed.

3.6. Air-filled root porosity

Wetland plants normally have well developed aerenchyma in their roots, which may be connected to aerial parts of the plant (Crawford, 1989). Many non-wetland plants can also form air channels in their cortex, under conditions of poor aeration (Drew, 1990). Such air channels are normally not connective with shoot aerenchyma. Erdman et al. (1988) described that no shoot to root continuity of air channels exists in wheat, but that the coleoptile sheath allows oxygen to reach a point close to the basis of nodal roots. The main function of cortical air spaces may be to bridge sites of better and poorer aeration along a single root axis. The distance which can be bridged depends on respiration rate of root tissue, air-filled root porosity and on the conductance for oxygen of the exo- and epidermis root (Luxmoore et al., 1970; De Willi-

gen and Van Noordwijk, 1989). Reliable methods for measuring air-filled root porosity are available (Jensen et al., 1969; Van Noordwijk and Brouwer, 1989) and the adaptability of roots to poor aeration as a function of age and physiological state can be investigated (Laan et al., 1989). The conductance of tissues between cortical air spaces and the outside world is more difficult to quantify as yet. A related question is the connectivity of air channels between main axes and branch roots. Anatomical evidence suggests that a branch root, originating from the stele of a main axis, normally has a fully developed epidermis inside the cortex of the main axis and may even be covered by cell walls of collapsed cortical cells. Semi-quantitative evidence on the effects of such a barrier on oxygen transport can be obtained by using redox dyes (Crawford, 1989; Laan et al., 1989).

Figure 8 shows a method to visualize oxygen transport in roots. A plant is fixed to a plate and the root system is spread out between pins under water (Fig. 8A); oxygen is removed from the root surface by applying nitrogen gas. A water vessel (aquarium) is prepared with a redox dye, e.g., Cristal-Violet $C_{25}H_{30}ClN_3$ using 10 mg l^{-1} , applied from a concentrated stock solution prepared in ethanol. The water is de-oxygenated and the dye is brought into the

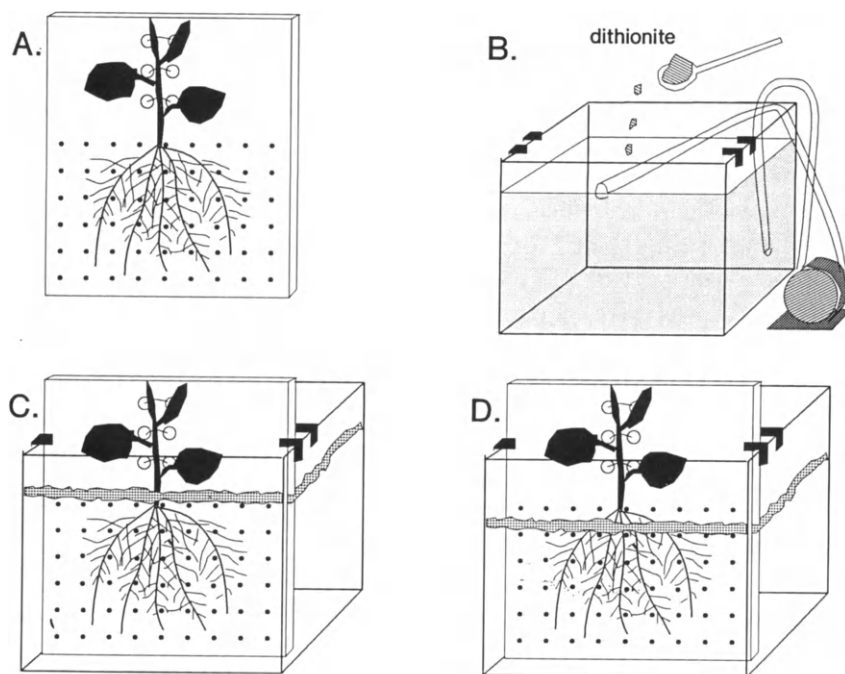


Fig. 8. Redox dye method for studying oxygen leakage out of roots as indication of functioning air channels; further explanation in text.

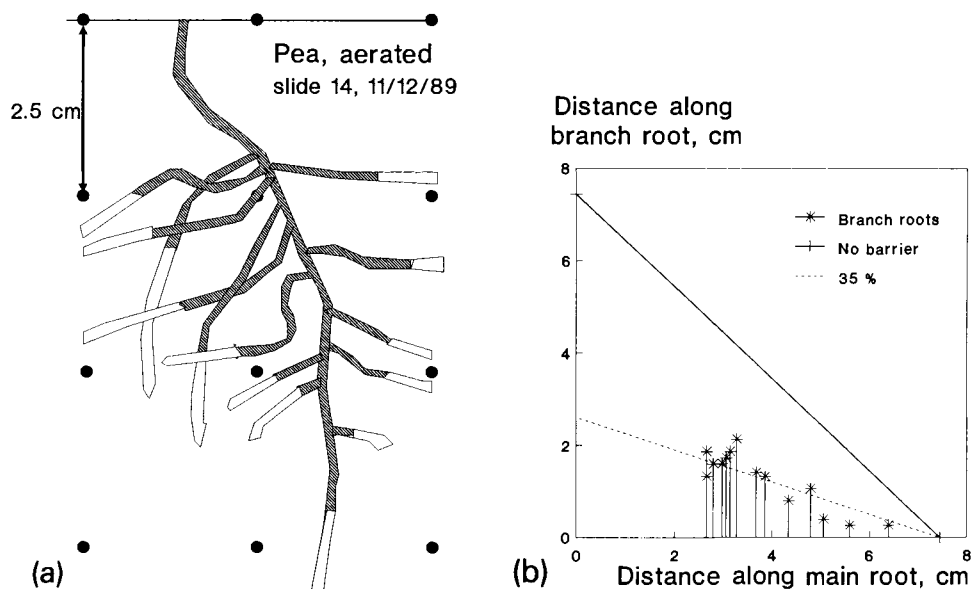


Fig. 9. (a) Blue pattern on a branched pea root in the redox dye test shown in Fig. 8. (b) Distance between water surface and furthest blue color on roots (explanation see text).

colorless reduced stage by adding sodiumdithionite ($\text{Na}_2\text{S}_2\text{O}_4$) and mixing the solution with an aquarium pump, without disturbing the surface (Fig. 8B). The plate with the root system is now placed into the solution; the amount of water in the vessel can be chosen such that either the shoot base is just under the water level ("high tide", oxygen in the roots has to come from the shoot) or that the basal part of the roots is exposed to air ("low tide", Fig. 8D). The water level is closed off using plastic grains as a mulch, to reduce oxygen enrichment of the water. A blue color will develop on the root surface where oxygen is leaking out of the epidermis. The pattern of blue coloring can be recorded on photographs or drawings. Maximum color intensity is reached in about 2 h, but the extend of the blue zone is not time dependent.

Figure 9a shows the dye pattern observed on a branched pea root. In Fig. 9b the distance between the transition blue-white and the atmosphere is given, separated in a distance along the main root (X -axis) and branch root (Y -axis). Points would fall on a -45° line if no barrier existed between main and branch root and transport in the branch root was as effective as that in the main root. The pattern observed suggests that a partial barrier between main and branch root does exist. Observations on other crops, e.g. rice, suggests that in large parts of older roots the root exodermis is impermeable for oxygen, increasing the roots effectivity as air channel, but decreasing the possibility of oxygen uptake in favorable zones as well.

3.7. Dynamics of root growth and decay

Minirhizotrons offer a flexible method of studying the dynamics of root growth and decay under field conditions (Lussenhop et al., 1992). By recording the fate of individual roots or at least on the number of roots which died and the number which appeared new in each observation interval (Fig. 10), information can be obtained which is much more reliable than that of sequential destructive sampling. For studies of the effects of soil structure on dynamics of root growth and decay, however, one has to be careful that the observation plane does not introduce artefacts. Incomplete contact between the minirhizotron wall and the soil may allow roots to grow in a gap, with little relation with the surrounding soil. Condensation of water on the minirhizotron wall, caused by differences in temperature between soil and air inside the minirhizotron, is a clear symptom that a gap exists between minirhizotron and soil. Gijsman et al. (1991) described an inflatable system which avoids such a gap. A comparison between fixed-wall and inflatable minirhizotrons may allow a test of the hypothesis that roots growing in macropores (cracks) are more susceptible to herbivory and may live shorter than roots which created a new channel in the soil matrix.

4. EPILOGUE

A combination of the methods described here may allow a better under-

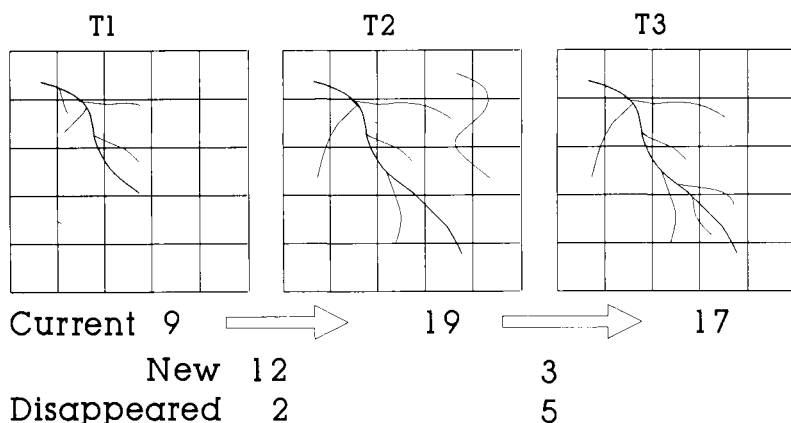


Fig. 10. Analysis of root growth and death from sequential root images, e.g., obtained by minirhizotron technique. For each interval the number of new intersections between roots and lines of a grid is counted, as well as the number which has disappeared. Actual root intensity, root growth and root disappearance can then be expressed as fraction of the cumulative number of new root intersections for the whole season. If at one date a destructive root sampling yielded reliable estimates of actual root length density, absolute root production estimates can be obtained.

standing of processes by which soil structure affects root growth and functioning and of differences between crops in susceptibility for poor soil structures. Still, for practical applications no real alternatives exist as yet for the synthetic view on soil structure of spade tests and visual rating systems (Taylor et al., 1991).

APPENDIX. STEPS IN DERIVING THE ROOT POSITION EFFECTIVITY RATIO, R_{per}

(1) Digitize a map of the root distribution in a horizontal or vertical plane and derive a list of the X, Y coordinates of roots.

(2) Introduce "barriers" in the root map, e.g., representing incomplete root-soil contact.

(3) Derive the frequency distribution of distances from points in the soil to the nearest neighbour root, e.g. by stepwise dilation of root points, allowing for the root barriers, and measurement of the area (number of pixels) after each step; a relative frequency distribution is obtained by dividing all values by the number of roots on the map.

(4) Derive an "annulus fraction" $f_{a,i}$ representation of these nearest-neighbour distances by dividing the fraction of soil in each distance interval R_i to R_{i+1} by the area of a circle annulus, $\pi(R_{i+1}^2 - R_i^2)$; the result can be presented graphically as in Fig. 6.

(5) Determine the circle frequencies, $f_{c,i}$ by "cutting the pie", i.e., for $j=0$ to n calculate:

$$f_{c,n-j} = f_{a,n-j} - \sum_{i=n-j}^n f_{c,i}$$

(6) Derive the G -sum $\sum f_{c,i} G(\rho_i)$, where the G -function is based on R_i (eq. 3).

(7) Find (by iteration) ρ^* for which $G(\rho^*) = \sum f_{c,i} G(\rho_i)$ and the corresponding L_{rv}^* .

(8) $R_{\text{per}} = L_{rv}^* / L_{rv}$.

A programme for steps 2 to 8 has been developed for a Quantimet 570 image analyzer, and is available on request.

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The role of biology in the formation, stabilization and degradation of soil structure

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ABSTRACT

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Soil structure is defined as the arrangement of particles and associated pores in soils across the size range from nanometres to centimetres. Biologic influences can be demonstrated in the formation and stabilization of aggregates but it is necessary to distinguish clearly between those forces or agencies which create aggregations of particles and those which stabilize or degrade such aggregations.

The formation of soil structure involves the physical forces of shrinking and swelling created by changes in water status of soils, freezing and thawing, tillage, or by movement of the larger biota in soils. Expansive properties of soils are controlled by the clay content. Thus changes of structural organisation are minimal in sands and maximal in clays. Plant roots, earthworms and other macrofauna large enough to move soil particles create pores recognisable by cylindrical shapes and smooth curved surfaces. Various visual and microscopic techniques aided by dyes are available to demonstrate the extent of biovoids in soils.

Biology plays a major role in stabilization of soil structure. The major factors vary depending on the scale of soil structure. At larger scales plant roots and associated hyphae can be seen to enmesh soil particles by acting as a “sticky string bag”. At the microscale the influence of mucilages from roots, hyphae, bacteria and fauna such as earthworms can be shown by a range of microscopic techniques to be involved in stabilizing smaller aggregates and the linings of biopores. Techniques include optical and fluorescence microscopy, scanning electron microscopy including EDAX, transmission electron microscopy using heavy metals or other electron dense staining techniques for specific chemical compounds, and computer aided tomography. The microscopic techniques can be used on individual aggregates, stabilized soils, sections or separates of soils.

Both microflora and fauna are involved in the degradation of stabilizing agents. Fauna may comminute roots and hyphae which stabilized larger aggregates and microorganisms utilize mucilaginous stabilizing agents as an energy source resulting in a slow breakdown of structural stability. Such effects can be established by combinations of studies of aggregation including microscopy. Further destruction of structure is caused by tillage and compaction by vehicles and animals.

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INTRODUCTION

The role of biology in the formation and stabilization of soil structure is being recognised increasingly by scientists and there is growing interest in managing soil biota to develop desirable soil structure and to minimise the use of machinery for production of tilths and optimum seed beds.

Soil structure has been defined simply as the arrangement of particles and pores in soils. To this definition must be added the stability of the structure or architecture of the soil because structure is not static and changes with water content and other agencies of stress which may be applied to the system. Structure needs to be defined across nine orders of magnitude (Waters and Oades, 1991) but descriptions at any one scale need to be integrated into the properties of the whole soil otherwise the scientific endeavours will have little value in the field. Similarly the size range of soil biota which influences soil structure is enormous, from soil microorganisms and their biopolymers, through arthropods and Collembola to moles, rodents and wombats, to large plants such as trees.

There is a good deal of confusion in the literature with respect to the formation of aggregates and pores, and their stabilization. In many cases a particular structure is formed by one process and stabilized by another. Sometimes the formation and stabilization occur simultaneously but often formation precedes stabilization. In this review the simple definitions in Table 1 will be followed.

The major forces involved in the formation of structure are the physical forces created by wetting and drying which increase with the clay content of soils. However, roots and the larger soil organisms also create soil structures in both direct and indirect manners. The major role for biology is in the stabilization of soil structure, although in some undisturbed soils faecal pellets may dominate the upper horizons.

The greatest influence on soil structure—both creation and destruction, but rarely stabilization—is caused by tillage, by traffic and by hooved animals.

In this review an attempt is made to discuss those techniques which allow

TABLE 1
Definitions used in this review

Soil structure	the arrangement of particles and pores
Structural stability	the stability of a particular arrangement to internal and external stresses
Formation	of an arrangement of particles and pores
Stabilization	of an arrangement of particles and pores by organic or inorganic materials
Degradation	a detrimental change in structure for, e.g., aeration, water movement, root growth, etc.

structural features in soils created by biological and physical factors to be recognised, and where possible quantified so that biota can be managed to produce an optimum stable soil structure *for crop production*.

FORMATION OF SOIL STRUCTURE IN SANDS, LOAMS AND CLAYS

Before discussing biology and structural features we need to establish a few basic principles with respect to modern views of soil structure. We need to consider sands, loams and clays separately, particularly if we wish to compare biotic and abiotic influences on structure.

Soil structure as defined in the introduction is a broader concept than aggregation and includes aggregation. Particles in a soil may be single-grain mineral particles, e.g., quartz, or aggregations of single-grain particles into compound particles which are generally referred to as aggregates or peds. Other terms such as granules or crumbs have implications for porosity and strength. A sand has structure because it has a pore size distribution created by the size and the packing of sand grains. This structure can be changed by altering the packing of the sand grains by tillage or compaction or rearrangement by soil animals. The structure is not altered significantly by drying and wetting cycles because the shrink–swell capacity is virtually zero. Binding of sand grains together will thus depend on biological factors.

In loams the cohesive nature of clays and the shrink–swell capacity associated with colloidal particles creates aggregates during drying and wetting cycles. The existence of an aggregate infers that the cohesive forces between the particles within an aggregate are greater than those between the aggregates. In situ the aggregates may be separated by voids or are defined by planes of weakness which may not be obvious unless the system is stressed mechanically. This can occur internally due to drying and wetting or externally by tillage. The greater the clay content, the greater the shrink–swell capacity and the more vigorous the cycles of structural formation during dry–wet cycles. Plants play a major role in structural development of loams because they influence the rate, extent and spatial development of the drying phase. Root systems also play a major role in stabilization of the structure created. Soil fauna also create aggregates as faecal pellets and both biotic and abiotic factors are important in loams (Table 2).

Maximum development of aggregation occurs in smectite-rich clays in Vertisols and Mollisols. In Australia some clay soils exhibit the desirable structural feature of “self mulching”. This involves the development of a friable, granular soil structure in the top few centimetres of soil after only a few drying and wetting cycles. Even a severely puddled soil will regenerate this desirable structure after two or three wet–dry cycles. Attempts to quantify this property have been described by Grant and Blackmore (1991). In such clay-rich soils the creation of structure is dominated by the behaviour of the clays and as far

TABLE 2

Biotic and abiotic influences on soil structure

	Sand ^a (< 15% clay)	Loam ^a (15–35% clay)	Clay ^a (> 35% clay)
Shrink–swell capacity	Minimum	Important	Maximum
Abiotic aggregate formation	Minimum	Important	Maximum
Biotic influences	Aggregate stabilization and degradation	Aggregate stabilization and degradation	Minimal (biopores?)

Determined by the coefficient of linear extensibility?

as the top few centimetres of these soils are concerned biological factors are not important for either structural formation or stabilization. Similar tith-mellowing processes are utilized by farmers in northern latitudes by exposure of soils to freeze–thaw cycles.

The precise definitions of sand, loam and clay will cause a good deal of discussion. Pedologists will use clay contents. Heinonen (1982) and Horn (1990) have suggested that at least 15% clay is needed for the abiotic development of aggregation in soils especially those which have been compacted. Thirty to 35% clay is the content usually required for the textural definition of clay. However, the precise clay content as determined by the classical procedure is not important and a measure of shrink–swell capacity such as the coefficient of linear extensibility (COLE) would be a more useful parameter to differentiate between sands, loams and clays for the purpose of structural studies.

The importance of biology in structural formation is greatest in soils with low shrink–swell capacity and minimal in self-mulching clays. Biology plays a major role in stabilization of soil structure in sands and loams, providing sodicity is excluded. A basic assumption is that the soil undergoes drying–wetting cycles. If not, the biotic factors may be relatively more important.

ABIOTIC FORMATION OF SOIL STRUCTURE

In sands any clay present will be drawn into interstices between larger mineral particles by water menisci as the soil is dried. This may create aggregation of clay particles in the micron range. Whether or not these new aggregates remain stable when the soil is rewetted will depend on the severity of drying, the shape and packing of the particles and the electrolyte environment. In loams the cohesive behaviour of the clays becomes a dominant factor. On drying shrinkage occurs and creates tensile stresses which will eventually lead

to the development of cracks along planes of weakness thus creating aggregates. The development of desiccation cracks in a "uniform" soil has been illustrated by Dexter (1988), see Fig. 1.

The distances between cracks is controlled by the distribution of planes of weakness and is crucial to the development of soil structure. Cracks will appear where the soil has low tensile strength which is where the soil is wettest. One factor controlling cracking patterns is thus the uniformity, or lack of it, during drying. The drying of surface soils is often controlled by plant roots and major biopores which serve as sinks for water. Within the root zone the soil is dried and shrinkage causes cracks to appear in wetter regions not yet influenced by water uptake from the roots. This explains the common observation of cracks running between and parallel to plant rows of cereals and maize. Under plants not sown in rows, e.g., pastures, the even distribution of roots will cause smaller more frequent cracks with no specific orientation. The result is a well-aggregated granular soil to the depth of maximum root development.

One consequence of structural formation by shrink-swell processes is the tendency for smaller denser aggregates to be formed. It has been claimed that both roots and earthworms have aided compaction during their passage through soils but since an impedance of 3 MPa limits root growth by $\sim 80\%$

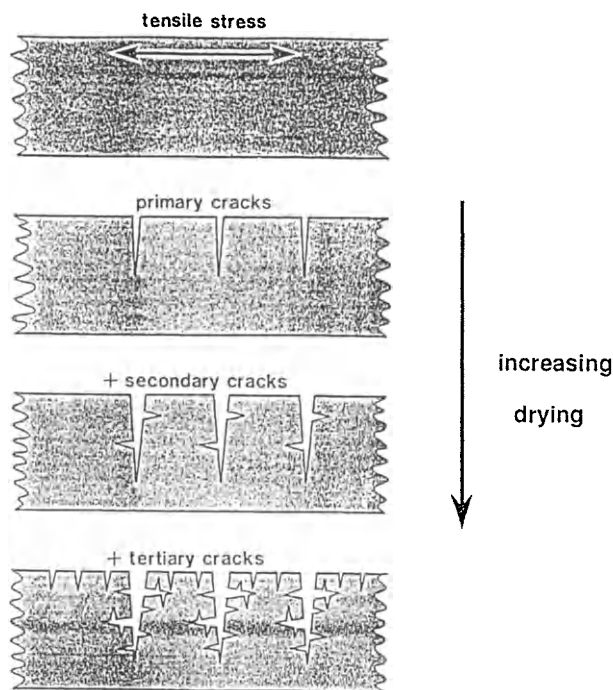


Fig. 1. Development of soil structure by shrinkage on drying (after Dexter, 1988).

(Greacen, 1981) and the forces exerted by earthworms are unlikely to exceed 0.2 MPa (McKenzie and Dexter, 1988) the biologic effects are unlikely to be significant except in very wet soils.

Methods for assessing soil structure do not usually differentiate between biotic and abiotic factors responsible for creating structure. A range of methods are available with an interesting mix of approaches by pedologists and physicists, with more recent influences by biologists. A concise overview of methods is available with a physicists bias in the review by Dexter (1988).

AGGREGATE HIERARCHY AND THE EXCLUSION PRINCIPLE

Aggregation in loams and clays leads to concepts which can help in determining the importance of the biologic cycle and organic materials in soil structure and its stability. One concept is that of aggregate hierarchy. Aggregation of a range of different soil particles of different sizes could occur such that large aggregates contained all soil components in a completely random fashion. The large aggregate would have no planes of weakness so that when it disintegrated all the elementary single grain particles including clay plates and crystals would be released immediately. A simple approach to such a phenomenon would be an aggregate formed by the drying of a strongly sodic soil with no biologic inputs. When stressed by rewetting the clay would swell and disperse and all larger particles would fall apart. However, this tends to be the exception and more commonly large aggregates disintegrate under stress to yield smaller aggregates. This process may be repeated several times before the soil is broken down to its textural constituents. This concept of aggregate hierarchy has been described by Kay (1990) and Waters and Oades (1991) in the figure indicating that several hierarchical orders exist in some soils, e.g., clay microstructures represent a first order measured in terms of microns. A second hierarchical order was termed microaggregate, $\sim 100 \mu\text{m}$ in diameter, and a third order, macroaggregate with diameters of several millimetres. Larger aggregates were termed clods (tens of centimetres) and are generally regarded as an undesirable result of cultivation of wet soils.

The concept of aggregate hierarchy is illustrated in Fig. 2 which shows a small group of ten small particles which form an aggregate because they are in relatively close contact. The secondary aggregates are themselves grouped to form a tertiary compound particle and so on. One consequence of this aggregate hierarchy is the porosity exclusion principle (Currie, 1966; Dexter, 1988) which shows that smaller aggregates should have the lowest porosity and the greatest contact between particles. Thus the tensile strength of smaller aggregates will be greater than that of larger aggregates (Braunack et al., 1979; Hadas, 1987). Such aggregates in the soil will disintegrate in a stepwise fashion and not catastrophically. Oades and Waters (1992) demonstrated aggregate hierarchy in a Mollisol and Alfisol but not in an Oxisol. The hierarchy

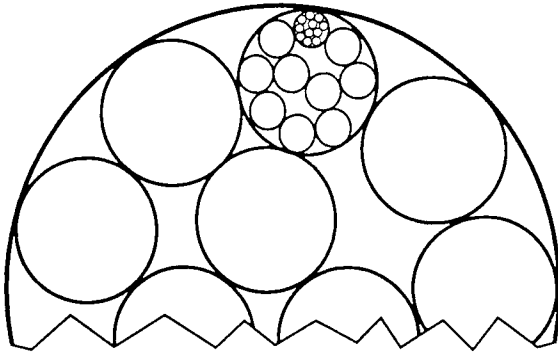


Fig. 2. The concept of aggregate hierarchy.

was considered to be due to root systems and was exhibited to the fullest extent in a Mollisol which had been under prairie grassland for hundreds of years. The structure of the Oxisol was stabilized by oxides as well as organic materials and hierarchy was not demonstrated in this soil which was very stable but eventually broke down to release clay and silt with no obvious intermediate stages.

In an ideal soil aggregate hierarchy would occur in even steps as illustrated in Fig. 2 and the morphology of the aggregates of each hierarchical order would be similar at different scales. In this situation the fractal approach to soil structure as described by Bartoli et al. (1991) and Young and Crawford (1991) should prove useful. Bartoli et al. suggested that self similarity did exist in silty and sandy soils. Where root systems are involved in stabilizing aggregates in loams and clays aggregate hierarchy appears to exist, but the stabilization by the biological agencies is of particular sizes of aggregates which have different morphology as well as scale. One might speculate at this stage that aggregate hierarchy exists in soils as a legacy from a long history of exploration by roots, particularly from grasses, and that it will not apply to very young soils such as the polders, or perhaps to soils where inorganic cements are dominant, e.g., Oxisols.

BIOTIC FORMATION OF SOIL STRUCTURE

Structure can be formed by the creation of aggregates or pores. The formation can be direct as when larger fauna ingest soil and produce excreta in the form of casts or pellets and the formation of biopores by roots, earthworms, termites, ants, spiders and the larvae of various beetles and moths. In general the mesofauna are not considered important in the formation of structure in arable soils because they are too small to move most soil particles. The best quantitative data available are those of Didden (1990) who showed that about

one third of enchytraeid worms studied in Dutch soils contained mineral grains. However, calculations indicated that it would take at least 100 years for these small worms to turnover 1% of the 0–40 cm layer. However, the mesofauna in conjunction with the larger fauna could well be beneficial in enhancing and stabilizing the pores in which they live. This may also be true of mites and Collembola which rely on earthworm channels for descent into the soil with the onset of drying (Malinda et al., 1982).

In general the mammals which burrow in soil do not have beneficial effects especially in arable soils and usually create problems such as in dam walls. For the activities of animals in soils the review of Hole (1981) should be consulted.

The biotic influence on formation of soil structure can be indirect such as the impact of root systems on drying in loams and clays. Roots grow mainly in wet soils with low tensile strengths and even then prefer to grow in pores rather than through aggregates. The root system dries the soil and it is of interest to compare the potential drying of the soil by dicotyledenous plants compared with monocotyledenous plants such as grasses. The latter have numerous fine roots which dry the soil at countless sites thus creating many non-oriented cracks which give rise to the beautifully granular, crumb structure associated with old grassland soils, e.g., Mollisols before cultivation. It is difficult to separate the biotic and abiotic effects in loams and clays and there are few quantitative data on the role of roots in forming soil structure. The subject is complicated because roots may form and stabilize structure simultaneously. Unfortunately there have been few experiments which aimed to define the most beneficial plants with respect to structure formation and stabilization. Those which have, indicate that productive grasses are most efficient, e.g., Tisdall and Oades (1982) and Grevers and De Jong (1988). A challenge for sustainable agriculture is to identify those plants which are most efficient in forming stable soil structure and to incorporate them into economic rotational management systems.

THE DEVELOPMENT OF BIOPORES

The larger biopores in soils are made by roots and earthworms and are usually cylindrical and very long.

It has been claimed that roots of annual plants can exert pressures up to 9 MPa but they do not grow under such conditions. However, larger roots, e.g., tap roots of dicotyledons and tree roots exert greater radial pressures as they expand their diameters. Certain plants, e.g., jack pines are noted for their ability to penetrate strong soils. Annual plants have a lesser ability to penetrate strong soil, but even so plants with tap roots have capabilities to penetrate strong layers to depth. The old root channels then become a thoroughfare for new roots. Large root channels can be recognised by a lining of resistant

bark, infillings of the remnants of the root decomposition and often soil materials from upper horizons, as well as new roots. Successive generations of tree roots may follow the same channels, often through rocks, for many years. The surfaces of old root channels may be sites for crystallization of gypsum and calcium carbonate and in poorly drained soils old root channels show staining of iron oxides as the iron solubilized by reduction and acidity in the rhizosphere is oxidised and precipitated. Root channels are recognised by observation and the various techniques available for investigating biopores.

A review on soil fauna and soil structure was recently compiled by Lee and Foster (1992) including a section on earthworm burrows from which the following summary is drawn. The burrows are constructed by exertion of radial pressures to enlarge the burrow diameter or by moistening the soil with saliva and then ingesting it. The pressures developed are less than 0.2 MPa. Thus earthworms require a structured soil in which pores can be widened during the construction of the burrow or a wet soil in which burrows are created by ingestion and casting of soil materials. Burrows are generally of two forms. Those of anecic species which are semipermanent individual systems with mainly vertical channels and those of endogeic species which burrow continuously producing horizontally oriented extensive linked networks. When earthworms are plentiful, their burrows are large enough to dominate the macroporosity in soils and play a major role in water infiltration and gaseous exchange. Water infiltration may be increased tenfold and sometimes considerably more in soils with high populations of earthworms compared with soils with few earthworms.

Earthworm burrows are recognised by their cylindrical shape, size and length. The burrow walls like the walls of rhizopores are often coated with oriented clay, humic materials, calcium carbonate and iron oxides.

METHODS FOR INVESTIGATION OF BIPORES

A range of techniques is available to study biopores to extend what can be seen in vertical and horizontal sections of soils. Volumes and shapes of biopores can be obtained by pouring liquids into the pore systems to produce "casts" from which the soil materials can be washed away.

Various materials have been used as listed below:

latex	Garner (1953)
polyethylene	Willoughby and Walsh (1972)
polyester epoxy resins	Jongorius and Heintzbergen (1975)
paraffin wax	Dexter (1976)
plaster of Paris	Fitzpatrick et al. (1985)

In most cases the impregnated samples have been sectioned by sawing and smoothed depending on the scale at which they will be studied. The sections

have then been examined optically and microscopically, photographed and subjected to various forms of image analysis which enables the classification of soil pores based on size, shape and orientation. To obtain the three-dimensional arrangement of pores by serial sectioning is very time consuming. Predicting structure in three dimensions from two-dimensional measurements, i.e., stereology, is a developing area of soil structural characterization (Ringrose-Voase and Bullock, 1984; Kretzschmar and Monestiez, 1987; Ringrose Voase and Nortcliff, 1987) as is the application of computer aided tomography to soil structural studies (Phogat and Aylmore, 1989).

Biopores have also been studied using dyes to study both infiltration rates and to observe the flow pathways of liquids in sections of the soil. This is an excellent technique to determine preferred pathways of water flow through soil systems in the field, but has limitations at small scales (Murphy et al., 1977a, b; Omoti and Wild, 1979; Kooistra et al., 1985).

The soil "peel" method has also proved useful in studying biopores. The method involves pouring a resin onto a soil surface which can be cut vertically or horizontally in the soil. When the resin has set and has been reinforced, if necessary, it can be peeled off bringing with it a soil surface fractured along planes of weakness. This surface can be viewed to count pores and to approximate their sizes and shapes and to a limited extent their continuity (Plas and Slager, 1964; Bouma and Hole, 1965; Smettem and Collis-George, 1985).

Rogaar and Boswinkel (1978) derived the three-dimensional arrangement of earthworm tunnels using binocular microscopy and X-ray stereo-radiography. Radiographs of 5 mm thick sections were developed into drafted interpretations of an earthworm chamber.

Other large voids in soils of semiarid and arid regions are made by ants and termites. Both ants and termites construct burrows and galleries which are often very extensive both laterally and vertically to the extent that they influence the hydrologic cycle. Their impact on soils is great in small areas where they build nests and mounds. Their impact on aggregation is not known but is likely to be limited. For information the reader is referred to Lee and Wood (1971), Lobry de Bruyn and Conacher (1990) and Lee and Foster (1992).

THE DEVELOPMENT OF AGGREGATES

Two main types of aggregates are formed by soil fauna: earthworm casts and faecal pellets.

Earthworm casts

Earthworms may ingest substantial quantities of soil materials which are then cast on the surface or in earthworm burrows. For temperate pastures and grasslands Lee (1985) estimated that on average earthworms cast 40–50 t

$\text{ha}^{-1}\text{yr}^{-1}$ on the surface which represents 3 to 4 mm. More material is cast below the surface. Greater activity may occur in some soils in the tropics and in warm temperate climates.

Earthworm casts are characteristically spherical or ovoid pellets from 1 to 10 mm in diameter depending on species and age, or paste-like slurries which are still rounded in form. There are numerous reports on the importance of earthworm casts (Lee, 1985; Lee and Foster 1992). In some soils the top 10 or 20 cm of soil is entirely casted material. It is also clear that earthworm casts can be more stable than other soil aggregates (e.g. Monnier and Jeanson, 1964; Van Rhee, 1977; and others). For example casts have been shown (a) to withstand 5 to 54 times more kinetic energy from raindrop impact before disintegration compared with soil aggregates (De Vleeschouwer and Lal, 1981), and (b) to contain more water stable aggregates (Lal and Akinvemi, 1983) and to have greater tensile strength than soil aggregates (McKenzie and Dexter, 1987). In all instances where earthworm casts have been dried before measurements were made they have been shown to be stronger and more stable than soil aggregates. Drying of earthworm modexi causes substantial shrinkage and hence problems with measurements of bulk density and porosity. It is reported several times that earthworm casts have lower bulk densities and higher porosities than non-casted aggregates. If this fact can be confirmed it raises two interesting questions. The first concerns the greater strength of aggregates with lower bulk densities and higher porosities. In most soil aggregates the reverse is true as outlined in the porosity exclusion principle. Two explanations can be offered. One is the greater content of clay and silt in casts compared to non-casted soil aggregates. The second explanation concerns the mixing of all soil particles in the gut of the earthworm (Lee and Foster, 1992), to produce dispersed clay (Shipitalo and Protz, 1988; Marinissen and Dexter, 1990). The thorough mixing and presence of dispersed clay may improve surface contact between particles and eliminate larger pores which would serve as planes of weakness in non-casted soil aggregates.

Generally casts or modexi are recognised by their shape based on their roundness or sphericity, and when fresh, lack of rugosity. Quantitative morphological studies of aggregates are now possible. We have the possibility to do this in the field by simple comparative tests or to make more sophisticated and quantitative mathematical descriptions using computers and image analysis.

A range of authors have commented that it was easy to recognise earthworm casts without describing the criteria involved. Descriptive classifications have been developed by Barratt (1969) where the size of "pelleted" materials and their shapes such as cylindrical or obvate were described. She used other terms such as rugose and spongey. Bal (1973), who introduced the term modexi for faunal excrements, suggested five morphological criteria: spherical, elliptical, cylindrical, platey and mitoid (threadlike). For rela-

tively fresh earthworm casts this system would be a useful basis for description and classification of modexi but is difficult to apply to casts during stages of degradation.

The problem becomes that of recognising sphericity or roundness. A first approach could be to take a chart of standard comparison shapes such as those used in field handbooks (e.g. McDonald et al., 1990). Simple rating of aggregates against such a chart would readily allow the shapes and curvatures of modexi to be recognised and quantified at whatever scale was appropriate.

More sophisticated approaches to quantifying the shapes of aggregates have been described by Dexter (1985) who concluded that the most sensitive single value measurement was the shortest aspect ratio. This is the ratio of the shortest to the longest diameters through the centroid. Radius spectra and curvature (Fig. 3) gave the most comprehensive information on shape. These methods have been applied to earthworm casts by McKenzie and Dexter (1987). Casts were photographed and tracings made of cast shape. The tracings of aggregate outlines were scanned by a TV camera connected to a digitizer and computer to determine the centre of the aggregate which was then divided into quadrants for further scanning.

From the digitized outlines three simple ratio methods were calculated and two mathematical spectral analyses made, a radius spectrum and a curvature spectrum. Both spectra allowed quantitative assessments of the greater roundness of modexi compared with other soil aggregates.

Further approaches to recognition and quantitative assessment of earthworm casts could be based on measurements of sphericity, roundness and rugosity. It is possible now to do this with software programs and PCs. Further work is needed on bulk density as a means of separating casts from other aggregates.

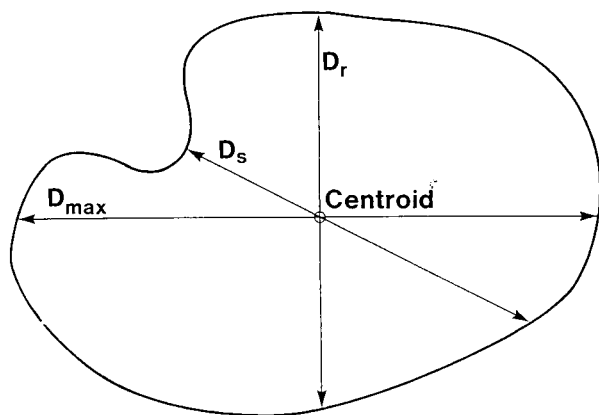


Fig. 3. Aggregate outline for description of shape (McKenzie, 1988). D_{max} = maximum diameter, D_s = smallest diameter, D_r = diameter at right angles to D_{max} through the centroid.

It is possible that such techniques with other more conventional approaches to aggregation may allow the relative importance of grass root systems and earthworms to be determined. For example Blanchart et al. (1989) claimed earthworms to be more important in forming aggregates in shrub savannas than grasses. Stewart et al. (1980) presented data indicating that earthworms were responsible for producing more aggregates than ryegrass. New Zealand experiences have indicated synergy between grasses and earthworms. In the presence of earthworms the beneficial effects of grass root systems was extended to a greater depth (Lee, 1985). It may well be that earthworms could be the major formers of soil aggregates in soils which are not subjected to severe wet-dry cycles and that grass root systems are the dominant formers of aggregates, through severe multi-point drying in soils with wet-dry cycles.

Faecal pellets

The smaller fauna do not play a major role in moving soil particles nor ingesting mineral materials but in certain environments such as forest soils, microarthropods, dominated by mites and Collembola are sufficiently active to influence structure by production of faecal pellets. The majority are saprophytic and produce faecal pellets which are mixtures of plant debris and humic materials. The pellets are usually < 1 mm diameter and can be recognised under the SEM by their roundness and smooth surface. With time they become densely colonized by fungi. In thin section viewed by TEM faecal pellets are readily recognised by the presence of densely packed bacterial cells (Lee and Foster, 1992).

THE STABILIZATION OF AGGREGATES

There are some soils in which structure is stabilized by inorganic materials such as oxides of aluminium and iron. This occurs in Oxisols and the biologic cycle although still performing the same role as in other soils is not the only stabilizing agent. In such soils it is not so important to maintain large organic inputs through the primary producers for soil structure. However, in tropical environments the very stable Oxisols may lose structural stability suddenly and catastrophically, as has occurred in some soils used for sugar cane production.

In most other soils biota play the major role in stabilizing structure especially in sands and loams.

For optimum aggregate stabilization there are three major requirements. Perhaps the most important is the photosynthetic input to the soil by the primary producers. This is the source of energy which drives the biologic cycle and there is no doubt that when the energy input is decreased by exploitive

management practices structural stabilization decreases creating a decline in what is normally considered to be desirable soil structure. This basic consideration is often forgotten in studies of soil structure because the inputs to soils are very difficult to measure and research workers are usually focused on specific mechanisms or organisms. The methodology involves long-term trials and development of models, e.g., the Rothamsted model (Jenkinson et al., 1991) and the Century model (Paustun et al., 1992) and the use of ^{13}C and ^{14}C as tracers to determine the cycling of carbon after inputs into soil by plants (e.g. Ladd et al., 1985). However, these approaches do not tell us how to recognise biologically stabilized structure.

A second major factor in structural stabilization is the form and distribution of the photosynthetic products added to soils. Are they added to the surface as litter, as large roots, distributed in numerous fine roots as in grasses, or in fact exuded from roots?

The third factor to consider is whether there are good conditions in the soil for roots, earthworms and other fauna so that structural stabilization is optimised.

In our quest for production we often limit carbon inputs to soil, we create external stresses and severely restrict growth of roots, animals, cryptogams and fungi.

Stabilization in sands

Stabilization of aggregates of sand particles involves the growth of higher plants, fungi and bacteria in the pore system between grains. The sand grains are then held together by (a) colonies of organisms and their mucilages (microbial aggregates), (b) roots and hyphae (root microbial aggregates) and (c) metabolic products from the decomposition of fragments of higher plants (Forster, 1979, 1990). The shape of the three types of aggregates were distinctive. Those stabilized by bacteria were spherical while those associated with roots, hyphae and plant fragments tended to have one long axis (Fig. 4). The absence of clays allows a straightforward development of stabilization not complicated by abiotic factors.

Stabilization in loams

The association of stable aggregation with grass was established long before the process was studied by scientists but we are now beginning to understand some of the mechanisms of the stabilization of aggregates by root systems, particularly the root systems of grasses (Tisdall and Oades, 1982; Oades, 1984, 1987).

In the Australian environment pastures are the only form of management which has been demonstrated to increase the organic matter status of soils

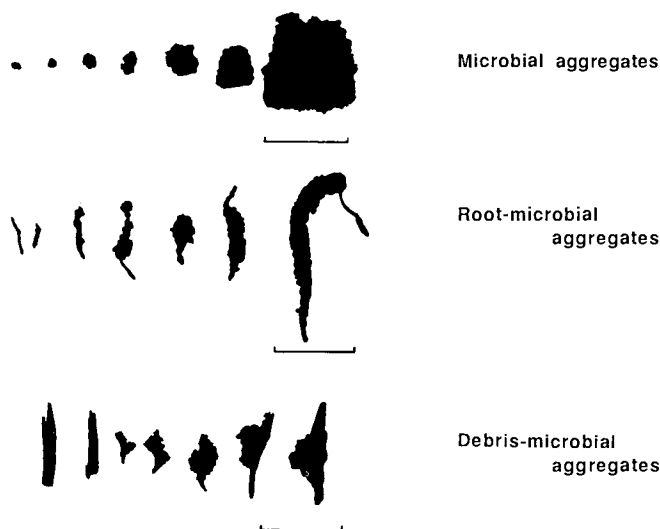


Fig. 4. Aggregate stabilization in sands (after Forster, 1990). Bar = 10 mm.

(Russell and Clarke, 1977). There is a positive correlation between the organic matter content of the soil and aggregation (Tisdall and Oades, 1982). Such correlations have been obtained by workers round the world. As stated earlier the grass root systems both form aggregates and stabilize aggregates simultaneously and it is not easy to separate the processes. In the absence of plants (long fallows) the wet-dry cycles continue, albeit not as vigorously as under pasture, and the stability of larger aggregates is lost. The difference is the lack of a growing root system with hyphae and rhizosphere so that even if aggregates are formed by tillage or internal stresses they are not stable to subsequent stresses. The methods involved in studies of aggregation and stabilization by root systems depend on long-term experimental trials containing rotations of plants or crops of interest, or farmers paddocks with reliable histories. The long-term effects of the root systems can then be studied using the various measurements of soil structure described by Dexter (1988). Observations can be made in the field on undisturbed soil samples and on separates of aggregates by the naked eye, by optical and electron microscopy, etc.

Another approach is to grow plants under controlled environments to study the formation of stable aggregates by root systems (e.g. Tisdall and Oades, 1979). To date, various plants have been compared which confirm that monocotyledenous plants are superior to dicotyledenous plants in stabilizing aggregates and that grasses are better than cereals. What is required is a systematic approach to determine the most efficient species for sands, silts and clays with controlled wet-dry cycles or under optimum water relations with and without earthworms and other fauna.

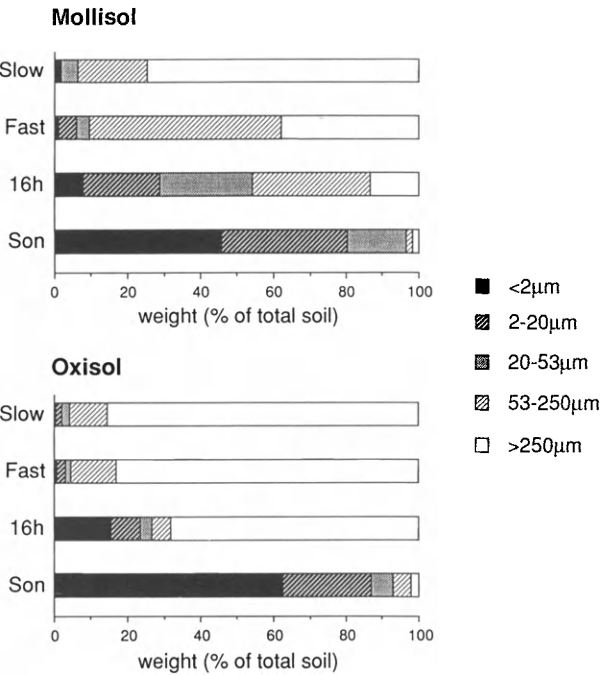


Fig. 5. Progressive disruption of aggregates from a Mollisol and Oxisol (Oades and Waters, 1991).

Methods for studying roots in soils and aggregates include root washing techniques either manually on sieves, or by using water jets to stir and disrupt soils to float off fine particles and roots so the roots may be sieved. Roots can be weighed or lengths measured by the method of Tennant (1975) or by image analysis procedures. There are problems with severity of washing and loss of root hairs. Similar procedures on a smaller scale can be used to measure the length of fungal hyphae in soil. The procedure of Tennant then requires the use of a microscope. For both roots and hyphae there are problems with separating living from dead. The use of various dyes can help in this respect.

For the study of intact aggregates SEM is the method of choice because the depth of field of binocular microscopes is limited. The SEM with EDAX can be used for identification of both inorganic and organic particles based on elemental analysis including heavy metal binding by organic materials. Recently in our studies of mechanisms for the stability of microaggregates and macroaggregates to establish whether or not aggregate hierarchy existed in soils we used a range of disaggregation procedures from gentle to vigorous followed by conventional characterization including SEM aided by EDAX and backscattered electrons (Waters and Oades, 1991; Oades and Waters,

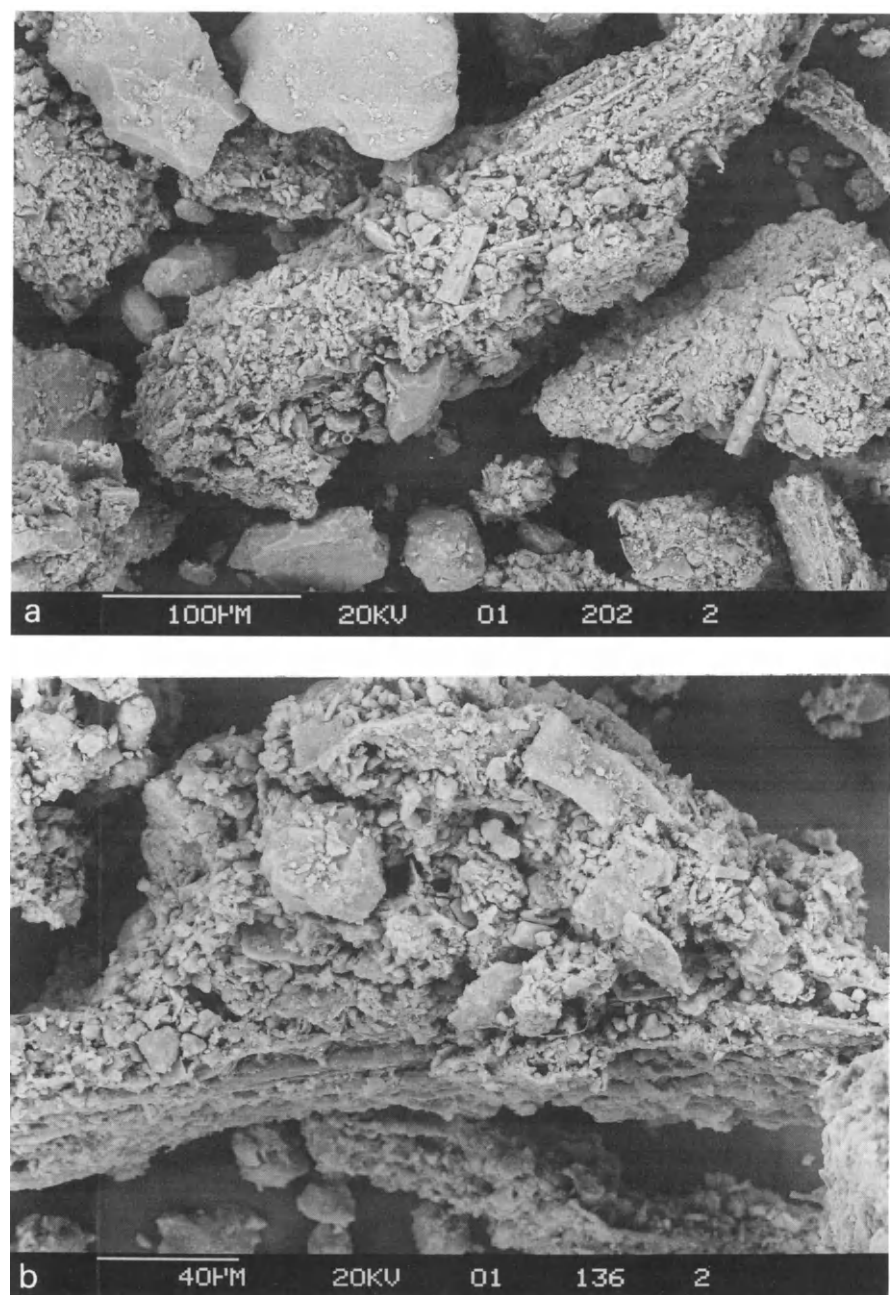


Fig. 6. SEM of encrusted plant fragments in aggregates of diameter 90–250 μm (reproduced with permission).

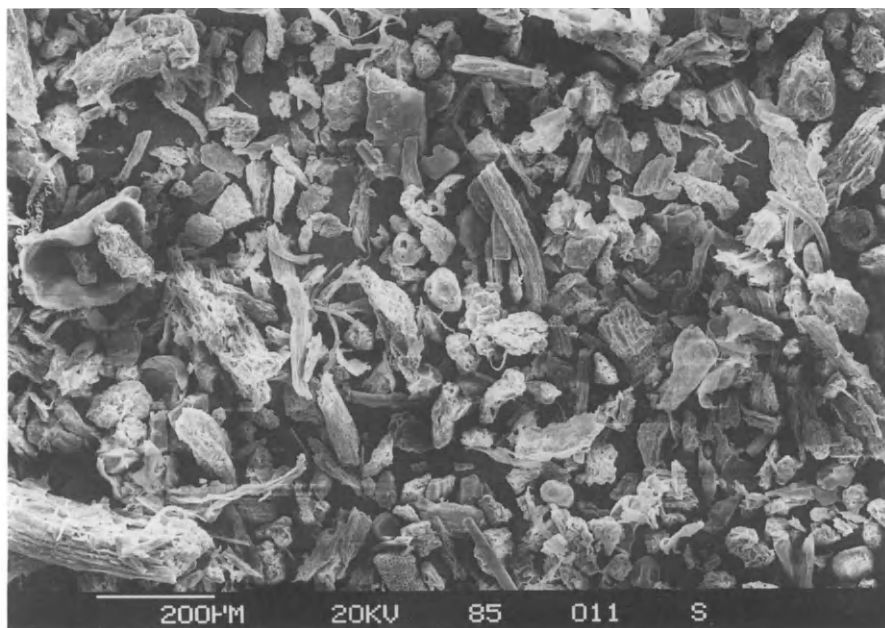


Fig. 7. Plant fragments freed from inorganic crusts by ultrasonic dispersion of aggregates of diameter 90–250 μm (reproduced with permission).

1992). The following summary outlines one approach to establishing the role of biology in aggregate stability.

The 0–10 cm horizons of a Mollisol and an Oxisol were subjected to slow wetting, fast wetting, mechanical shaking, and ultrasonic dispersion followed by fractionation into sizes from 2 mm to $< 2 \mu\text{m}$ (Fig. 5). The disaggregation patterns showed clearly that the Mollisol disintegrated in a stepwise fashion. Larger aggregates broke down to smaller aggregates before any significant release of fine particles. For the Mollisol fast wetting disrupted aggregates $> 250 \mu\text{m}$, the shaking disrupted aggregates $\sim 100 \mu\text{m}$ in diameter while ultrasonic energy disrupted aggregates 2–20 μm in diameter. This aggregate hierarchy was described by Tisdall and Oades (1982) and has been shown to exist in North American Mollisols (Elliott, 1986; Miller and Jastrow, 1990). It does not apply to all soils, for example, the Oxisol was shown to be very stable but when it was disrupted fine particles resulted and no step-wise breakdown was evident. Possible reasons for the stability of the microaggregates were illustrated by SEM of the particle size fractions released by the various disaggregation procedures. The SEM work showed clearly that many aggregates 100–200 μm in diameter had cores of plant debris. Some were completely coated with inorganic crusts but retained elongate shapes with length to width ratios greater than 2 (Fig. 6). When such aggregates were disrupted by ultrasonic energy obvious plant fragments were obtained by density separations

(Fig. 7). Smaller aggregates contained a few last remnants of plant debris or cavities left when the remnants had been completely utilized by microorganisms. The smaller aggregates were more spherical. Again as in Forster (1990) width to length ratios proved quite useful for identifying aggregates which owed their existence to remnants of plant debris.

Below 20 μm the SEM showed evidence of clay microstructure as illustrated by Oades (1987) but biologic features could not easily be found. The hierarchy is thought to be due to the stabilization of macroaggregates by roots and hyphae in the form of "sticky string bags". When the root systems die and are degraded, elongate aggregates of plant debris encrusted by inorganics result. The protection offered to the plant debris by the crusts slows down the decomposition of the debris and the microaggregates persist for perhaps hundreds of years. Such a concept should not apply to very young soils which have not had the chance to grow grass roots for long periods, e.g., young polders as indicated by Kooistra (1991).

Stabilization at scales below $\sim 20 \mu\text{m}$

The role of microorganisms and their metabolic products in soil structure has been reviewed many times. Various experimental procedures have been used to show that microorganisms, when supplied suitable substrates, will stabilize aggregates through filamentous structures, extruded biopolymers and particularly, extracellular polysaccharides (Burns and Davies, 1986). However, our concepts of the role of microorganisms in stabilizing aggregates at scales of 20 μm or less have improved over the last 10 years by studies of micromorphology using light and electron microscopy. The methods have been applied mainly to stabilized thin sections and surfaces but also to natural surfaces of aggregates. Sample preparations are crucial if the observations are to be made on unchanged biological materials with respect to shrinkage. Specialised techniques based on variations on apolar liquid replacement have been developed for drying samples without shrinkage. Staining procedures for roots, organisms and specific biopolymers have also been developed. These involve application of histochemical procedures using specific binding of heavy metals, including gold-coated lectins. The various submicroscopic techniques available for studying the preparations have been described by Bisdorf et al. (1990). Applications of micromorphology to soils to study structure and biota including quantitation of the results by image analysis have been described by Kooistra (1991).

Histochemical techniques to study biota and biopolymers in situ in soils have been developed by Foster in a series of papers over the last decade (e.g. Foster and Martin, 1981; Foster et al., 1983; Foster, 1986, 1988).

The preparation of samples to examine the detailed arrangement of organisms, biopolymers and inorganic materials in aggregates contains some art

with the science. The brief summary is gleaned from a series of Foster's papers. The preparation involves three major processes: physical stabilization to prevent movement of components during subsequent treatments, chemical stabilization to prevent loss of soluble components during various solvent exchange drying procedures, and staining with heavy metals to detect specific biopolymers. The latter reaction usually involves binding of the metal by a specific functional group.

The soils have been fixed by enclosure in agar or gelatin and chemically fixed with organic aldehydes such as glutaraldehyde, formaldehyde and acrolein or lanthanum hydroxide followed by osmium tetroxide. Tertiary butyl alcohol has been used for dehydration followed by embedding in Spurr's resin. The samples were viewed directly or sectioned using ultramicrotomy and diamond knives. The preparation of ultrathin sections of real soils remains a very expensive procedure because sand grains ruin diamond knives.

Osmium tetroxide reacts with phenolic hydroxyls, alkyl groups and sulphhydryl groups. Ruthenium red and lanthanum hydroxide enables detection of acidic polysaccharides in slimes and mucilages. Detection of neutral polysaccharides required more specialised treatments in which formaldehyde was used for fixation and postfixation by OsO_4 was omitted. Neutral polysaccharides were treated with periodic acid to convert 1,2 diglycol groups to aldehydes which then react with silver methenamine or thiosemicarbazide and silver proteinate. Further details and recipes can be found in Foster's papers particularly Foster and Martin (1981) and Foster (1988).

STRUCTURAL DEGRADATION

The term structural degradation assumes a change from an optimal structure to something less desirable for particular purposes. The same agencies may produce good structure and then go on to produce poor structure by the same mechanisms. For example wet-dry cycles will help to break down (mellow) a cloddy soil to a more desirable aggregate size for crop production. In the absence of a biological influence, i.e., no vegetation and associated organisms further wet-dry cycles will continue the disaggregation processes and may ultimately lead to complete incoherence of textural units.

The breakdown of aggregates is accelerated by cultivation, especially if the cultivation takes place when the soil is too wet, or too dry, i.e., the water content is distant from the plastic limit. Breakdown of the aggregates and the corresponding pores occurs according to the exclusion principle. Thus the larger aggregates breakdown first and it is the coarser pores responsible for drainage and aeration ($> 30 \mu\text{m}$) which disappear first. In a soil which has been under pasture the degradation is initially very rapid with a major decline in structure, as measured by hydraulic properties or aggregate stability in the first few years. It seems as though this initial rapid decline occurs as the root

systems are comminuted and the "sticky string bag" is disintegrated. The rate of decline in macroaggregation or macroporosity after a pasture is ploughed is thus very similar to the decomposition curve for plant materials added to soils and can be predicted based on mean annual temperatures (Ladd et al., 1985). Further structural degradation will then continue if cultivation persists and the input of carbon to the soil is limited. Eventually the microaggregates will also be disintegrated and the soil becomes very vulnerable to compaction and erosion.

Cultivation destroys the continuity of biopores by cutting them off at plough depth. Such pores will not then transmit free water. Cultivation disturbs the habitat of larger organisms and decreases their numbers.

Changes in soil structure below 20 μm are very slow. For example clay microstructure in soils is stable and is a characteristic of soils which is not likely to be influenced by management practices.

CONCLUSIONS

We now have available a range of techniques to measure soil structure at various scales. The techniques measure particles or pores or are based on transmissive properties for water.

There is a great need to apply these techniques to soils in the field where the history of soil management is well known. This requires the development of field tests, supported by laboratory work, otherwise there is a danger that there will be no links between field and laboratory.

Secondly we need the field data quickly so we cannot afford to use sophisticated laboratory procedures to obtain all our structural data.

There is a need to develop cooperative and multidisciplinary approaches to problems in soil structure. Again this will take time and requires a focus on soil structure and management and not on a particular disciplinary approach.

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Soil structure: carbon and nitrogen metabolism

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ABSTRACT

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Physical factors confer biological stability on organic matter in soils through the constraints they place on the opportunities for reactions between substrate and enzyme and/or decomposer organism; and, in the context of C and nutrient turnover through decomposer biomass, on the limitations they impose on the activities of predators on prey. The concept of protection afforded to substrates because of their location in micropores of sizes which deny access to decomposer cells, or to microflora in pores which deny access to microfaunal predators, has heightened interest in soil microstructure as a determinant of the availability of organic substrates for decomposition and of the rates of survival of decomposer cells.

Widely different methodologies have been used in studies which have attested to the importance of physical protection as mechanisms for stabilization of organic matter in soils. In this review we appraise some of the established methodologies and offer suggestions for extending their usefulness; and we briefly consider some new approaches where methodologies have not as yet been fully developed and whose potential for successful application to soils is unknown.

Studies considered include those which seek to characterize enzymes, substrates and organic residues, and their location in situ, in soil aggregates. Characterization of enzymes in situ derives from the use of electron microscopy in conjunction with cytochemical techniques. Characterization of substrates and residues in situ derives from the application of techniques of electron microscopy (SEM, TEM), and spectroscopy (NMR, IR), to soil aggregates or to fractions thereof. The successful development of methodologies combining electron microscopy and autoradiography could demonstrate directly within soil aggregates, sites of activity of specific microorganisms or enzymes in response to selected conditions of substrate addition.

Other studies seek to define more closely the relationships between decomposer activities and processes of C and N turnover and soil properties, especially those properties pertaining to soil microstructure. Water retention characteristics of a soil have been utilized to introduce substrates to different pore size locations within aggregates, in order to determine the effects of substrate location on C turnover. Physical fractionation techniques have been utilized to determine the location within aggregates of decomposer organisms, in order to assess the stability of fraction C and N in microbial biomass and organic residues resulting from the metabolism of introduced substrates.

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INTRODUCTION

Electron microscopy studies (SEM, TEM) have revealed the spatial complexity and heterogeneity of micro-environments within soil aggregates (Fig. 1) and the rhizosphere (Fig. 2), and have demonstrated sites of accumulation of organic residues of clearly cellular origin (tissue fragments, polysaccharides, etc.) (Foster, 1981a, b, 1985; Foster and Martin, 1981). The use of electron optical techniques has provided the visual evidence to reinforce conclusions drawn from other studies that physical protection mechanisms are important determinants of the stability of organic matter in soils (Figs. 3, 4 and 5) (Foster, 1981b, 1985; Ladd and Foster, 1987; Jocteur-Monrozier et al., 1991). Such mechanisms confer stability through physical isolation of substrates from decomposer cells and enzymes, partly as a result of their interactions with soil inorganic materials, partly from their associations with biologically resistant humic substances.

Some indications of the range of relevant experimental approaches involved, follow.

(a) Chemically-defined substrates (carbohydrates), relatively stable in soil

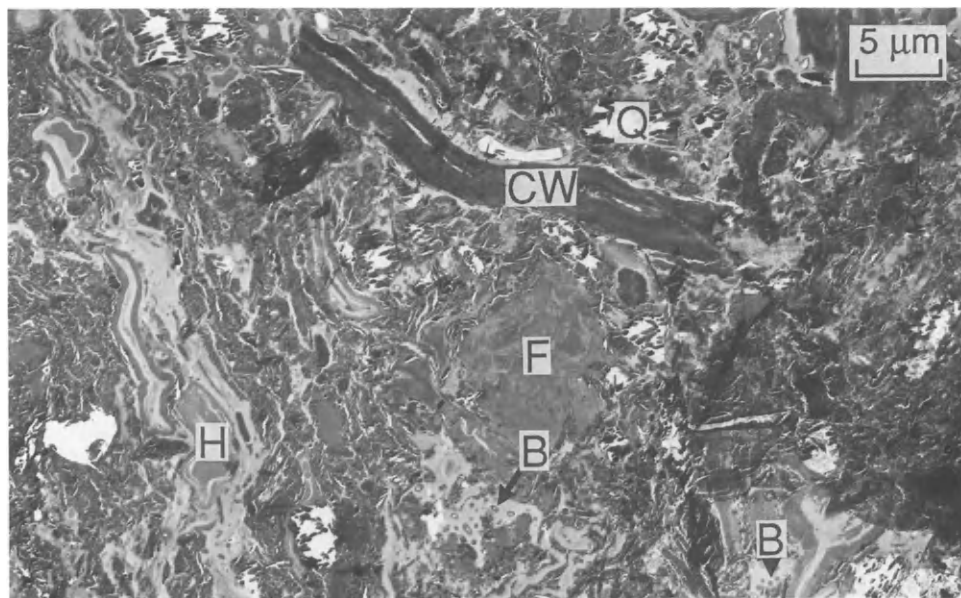


Fig. 1. Internal ultrastructure of an aggregate consisting of clay, quartz, cell wall remnants and colonies of bacteria, some in a wide void protected by narrow entrances. Figs. 1–5 and 8 are transmission electron micrographs of ultrathin sections of soil aggregates which have been enclosed in agar, fixed in glutaraldehyde/ OsO_4 , dehydrated through alcohol, embedded in Spur's resin. *B*=bacteria, *C*=clay, *CW*=cell wall remnant, *F*=faecal pellet, *H*=fungal hyphae, *M*=mucigel, *Q*=quartz. Arrows indicate deposits of heavy metal resulting from histochemical tests for enzymes.

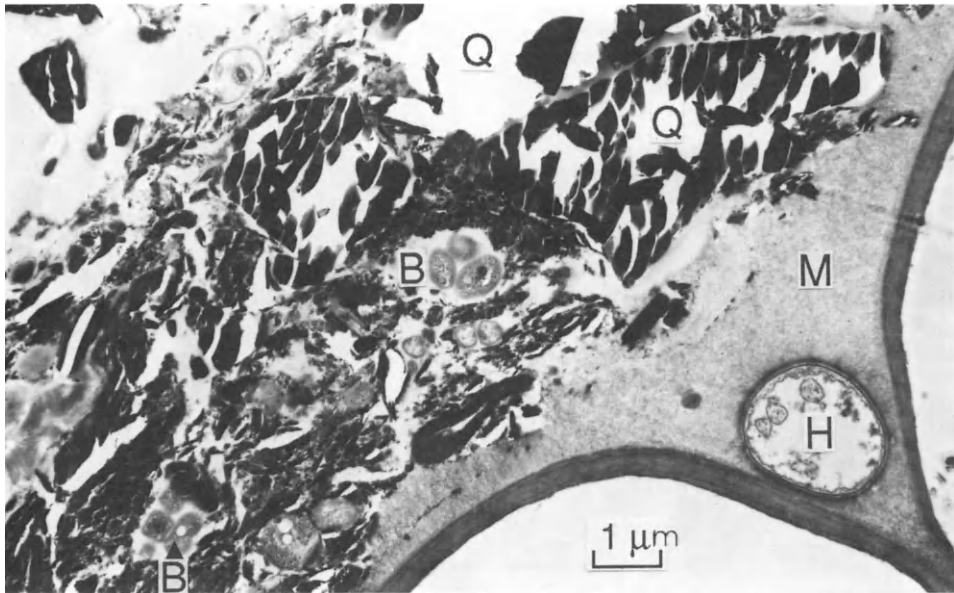


Fig. 2. *Paspalum* rhizosphere showing the dense clay soil, mucigel (*M*), and colonies of bacteria (*B*) which contain polyhydroxybutyrate (white) and polyphosphate (black) granules. Refer to Fig. 1 for details.

in situ, are readily decomposed when extracted from and readmitted to soil and incubated (Cheshire et al., 1974). Presumably their changed vulnerability to biological attack results from their changed locations within aggregates relative to decomposer organisms.

(b) Rates of evolution of CO_2 from soils amended with simple soluble substrates are most rapid during the first few days of incubation, due primarily to the metabolism of the added substrate itself (Sørensen and Paul, 1971; Van Veen et al., 1985). Within a few weeks rates decline markedly, even though high proportions of residues are accounted for in cells and chemically-defined cell debris and metabolites (Sørensen, 1967, Sørensen and Paul, 1971). The increasing average stability of the substrate-derived products of turnover are attributed to the development of conditions leading to physical separation of reactants.

(c) Flushes in biological activity (C and N mineralization) caused by gentle or severe disturbance of soils, or by intermittent drying and rewetting of soils, are thought to result mainly from changes in the relative locations of physically-protected substrate, enzyme and decomposer organism within the microfabric (Rovira and Greacen, 1957; Stevenson, 1956; Soulides and Allison, 1961; Sørensen, 1974; Gregorich et al., 1989; Van Gestel et al., 1991). It is inferred that such changes enhance the opportunities, albeit temporarily, for biological activity.

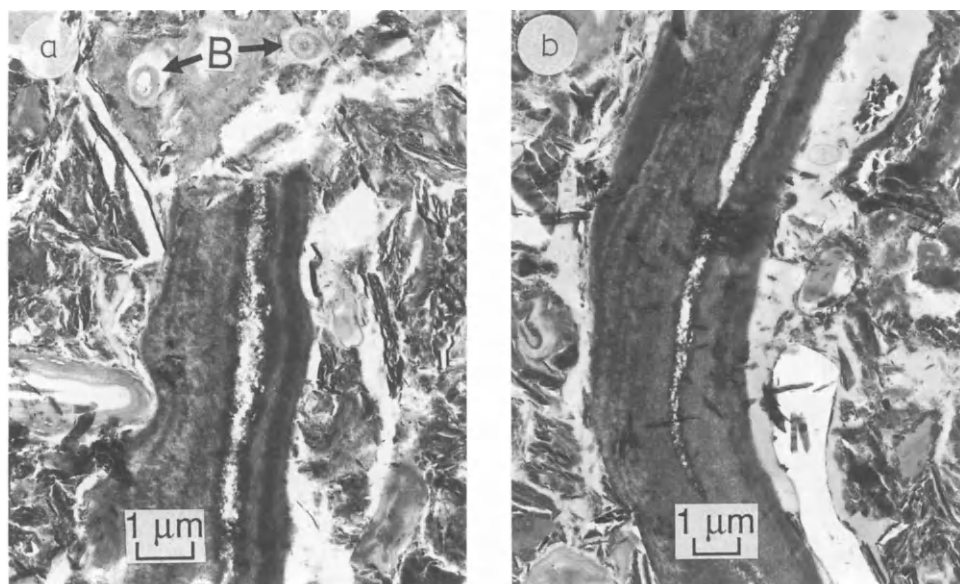


Fig. 3. Cell wall remnant which has been degraded by soil bacteria at its unprotected end (a), but not in the middle where it is physically protected by the surrounding dense clay fabric (b). Refer to Fig. 1 for details.

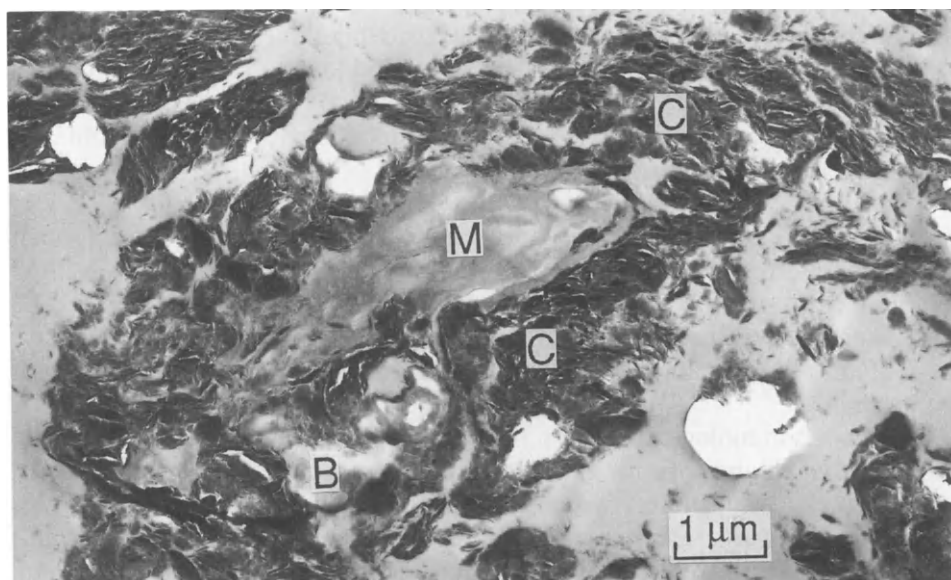


Fig. 4. Microaggregate consisting of a central core of mucigel physically protected by clay tactoids. Refer to Fig. 1 for details.

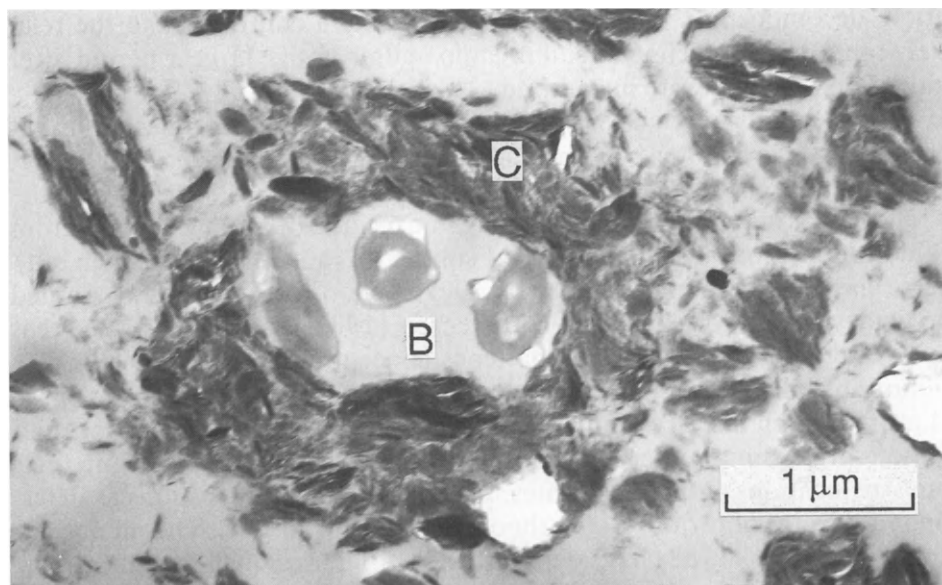


Fig. 5. Microaggregate composed of a small colony of bacteria enclosed in clay. Refer to Fig. 1 for details.

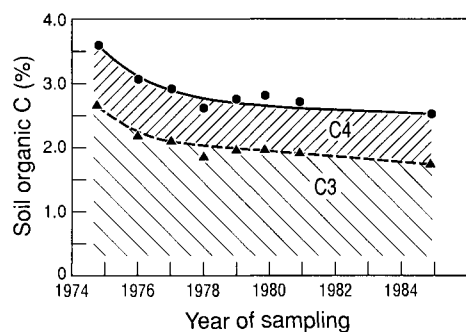


Fig. 6. Change in C3 and C4 carbon content of a Vertisol (0–15 cm) after continuously cropping with sorghum for 11 years. The soil was originally under brigalow scrub (C3) before planting to Rhodes grass (C4) in 1930.

(d) The effects of cultivation in decreasing soil organic matter levels are well documented (Tisdall and Oades, 1980; Elliott, 1986; Gupta and Germida, 1988; Van Veen and Kuikman, 1990). An interesting example is presented in Fig. 6 from studies by Skjemstad, who distinguished the rates of decline of soil organic matter derived from vegetation with C3 and C4 photosynthetic pathways. The soil was a Vertisol originally under brigalow scrub (a C3 plant), which was cleared in 1930 for growth of C4 pasture and then, after 40 years, was cultivated and cropped continuously with sorghum (C4). Despite little or no C3 carbon addition to the soil since 40 years before culti-

vation, decomposition of C3 carbon was the main contributor to the relatively rapid rate of decline of soil organic matter in the 11 year period after cultivation practices were introduced.

(e) The extents of C and N mineralization of added substrates are influenced by soil clay content (Kunc and Stotzky, 1974; Sørensen, 1975, 1981; Jenkinson, 1977; Ladd et al., 1981, 1985; Merckx et al., 1985). Most of the ^{14}C and ^{15}N of microbial biomass and organic residues which accumulate from the decomposition of isotope-labelled substrates in soils, is present in silt/coarse clay-size fractions (Ladd et al., 1977; Amato and Ladd, 1980; Ahmed and Oades, 1984; Cortez, 1989). Christensen (1987) has demonstrated that native and labelled soil organic matter is concentrated in the silt plus clay fractions of soil, irrespective of soil texture.

Limitations on the accessibility of substrates to decomposer microflora and of micro-organisms to microfaunal predators, by virtue of differences in their pore size locations within aggregates, invoke spatial considerations as determinants of rates of turnover of carbon and nutrients in soils. The pioneering studies of Hattori (1988) have demonstrated differences in the number, type, and behavioural responses of soil organisms according to their location within aggregates. The concept of spatial protection within aggregates of decomposer microflora (bacteria) from predation by larger microfauna, with significant consequences for C and N mineralization, has been explored in studies with soil microcosms (Elliott et al., 1980; Hattori, 1988; Kuikman et al., 1990, 1991) and electron microscopy (Foster and Dormaar, 1991; Jocteur Monrozier et al., 1991). Some such studies have quantified the conditions required for protection.

These studies complement others which have shown that

(a) Bacterial cells added to sterile soils survive better than those added to non-sterile soil, or to sterile soil amended with protozoa (Acea and Alexander, 1988).

(b) The survival of micro-organisms added to soil is related to soil clay content (Stotzky, 1966; Marshall, 1975; Heijnen et al., 1990). Bacteria and fungi coated with clay in earthworm casts are morphologically intact, whereas those without clay are reduced to empty cell walls (Lee and Foster, 1991).

Processes which physically isolate reactants constrain biological activity as long as the soil micro-environments remain undisturbed. Constraints may be lifted from changes induced at the molecular, particle or aggregate level.

Experimental evidence collectively supports the concept of physical (spatial) protection of substrate and organism in soils, a concept which has been embodied in several simulation models of C and N turnover (Jenkinson and Rayner, 1977; Van Veen and Paul, 1981; Van Veen et al., 1984; Verberne et al., 1990). More precise descriptions of the various mechanisms of protection are needed to assist in predicting the extent to which different treatments may remove physical constraints on organic matter turnover and nutrient miner-

alization. Quantification of the fundamental relationships between activity and soil microstructure will improve the accuracy of model outputs.

DECOMPOSER ACTIVITY AND SOIL PROPERTIES

Glucose decomposition and soil properties

Amato and Ladd (1992; Ladd, 1989) used 23 soils ranging in clay contents from 2% to 73% and amended either with ^{14}C -labelled glucose or plant material, to demonstrate unequivocally direct relationships between clay content of soils and the accumulation of ^{14}C -labelled organic residues. After about one year's incubation under controlled temperature-moisture conditions of the glucose-amended soils, the amounts of input ^{14}C remaining as total organic residues and as microbial biomass ^{14}C were directly correlated with clay contents (and CEC and total pore space). The slopes of the regressions of both total and microbial biomass residual ^{14}C on soil CEC were almost identical. Thus, whilst individual soils displayed some variation, non-biomass residual organic ^{14}C (i.e. the difference between total ^{14}C and biomass ^{14}C) statistically was independent of clay content and related soil properties.

Ladd et al. (1992) contrasted the behaviour of glucose-derived ^{14}C decomposing in a high-clay, strongly-aggregated Vertisol and a low-clay, weakly-aggregated Alfisol. Within a day of commencement of incubation until the end of the experiment (112 days), the Vertisol accumulated greater amounts of substrate-derived ^{14}C as biomass ^{14}C . Biomass ^{14}C was maximal in both soils after 2 days incubation, accounting for 51% and 32% of input ^{14}C in the Vertisol and Alfisol respectively, and declined to 28% and 10% of input ^{14}C in the respective soils by day 112. After the first 2 days to complete the metabolism of the added glucose, the amounts of input glucose ^{14}C residual as non biomass microbial products were approximately constant and similar in both soils (approx. 20–24% of added ^{14}C).

The relative gross rates of turnover of ^{14}C through the biomass and non biomass pools were determined for both soils as a function of decomposition time and assigned efficiencies of substrate utilization. Gross first-order rates of turnover of glucose-derived ^{14}C were calculated from measurements of $^{14}\text{CO}_2$ evolution rates and from net changes in biomass ^{14}C concentrations as described by Ladd et al. (1992). Figure 7 shows rates calculated over a 112-day incubation period, and for an assumed average efficiency of substrate utilization of 35% for both soils. Observed differences in ^{14}C behaviour in the two topsoils were due mainly to major differences in the calculated gross rates of decline of biomass ^{14}C , those in a low-clay Alfisol being 2.0–2.8 times faster than in a high-clay Vertisol (for any incubation time or nominated efficiency level). The relative differences between soils in the calculated gross rates of

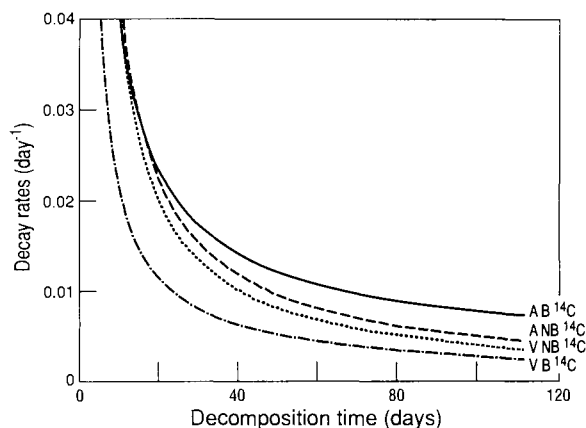


Fig. 7. Decay rates of biomass ^{14}C ($\text{B } ^{14}\text{C}$) and non-biomass ^{14}C ($\text{NB } ^{14}\text{C}$) in an Alfisol (A) and a Vertisol (V), calculated using an average substrate utilization efficiency of 35% (adapted from Ladd et al., 1992).

decline of non biomass ^{14}C to $^{14}\text{CO}_2$ and new biomass ^{14}C , were far less (1.0–1.2 times).

Plant residue decomposition and soil properties

The behaviour of glucose-derived ^{14}C in different soils was also observed generally when more complex substrates were involved. For example, for soils of neutral to alkaline pH, and amended with ^{14}C -labelled plant residues, total residual ^{14}C after 66 weeks incubation increased with increasing clay content of soils, and this was due principally to increases in the amounts of ^{14}C in microbial biomass (Amato and Ladd, 1992).

Mildly acidic soils, as a group, retained more residual organic ^{14}C from plant material decomposition than might have been anticipated from their biomass ^{14}C and clay contents. The reasons are unknown. The results may have reflected differences in the extent of the primary breakdown of the plant material itself, even after 66 weeks of incubation, or in the turnover of ^{14}C of decomposer cells, cell debris and metabolites.

Stabilization of biomass C in soils

In the study with 23 soils amended with either ^{14}C -labelled glucose or plant material, biomass ^{14}C (glucose-derived) residual in soils after 44 weeks decomposition was directly correlated with biomass ^{14}C (plant-derived) after 66 weeks decomposition (Ladd and Amato, 1988). Importantly, native biomass ^{12}C was also correlated directly with soil clay content and with biomass ^{14}C remaining from the decomposition of ^{14}C -glucose or ^{14}C -labelled plant

residues (Amato and Ladd, 1992). Thus, at times appropriately removed from periods of active metabolism, the amounts of "stabilized" biomass ^{14}C remaining in soils reflected the amounts of native biomass ^{12}C present. At such times the soil factors influencing microbial survival appear to be of overriding importance. The results support the concept, embodied in simulation models described by Van Veen et al. (1984, 1985), that soils have characteristic capacities to protect biomass C.

Experiments such as those reported above tend to emphasize the importance of clay content or related factors (cation exchange capacity, pore space) in determining differences in the decomposer activities of soils. They tend to imply that differences in the broad processes of C turnover lie, at least in the comparatively short-term, with the soils' capacities to stabilize biomass C, rather than with differences in the first order rates of decay of carbon in products of cell growth and metabolism.

There is a need to extend the range of soils tested for their capacities to accumulate residues from known substrates decomposing under standardized conditions. Such studies would (i) lead to a better appraisal of the relationships between clay content and the amounts of organic residues accumulating as biomass C and non biomass C, and (ii) better define the qualifying influence of soil pH (or other soil factors, e.g. aluminium in acid soils, calcium in alkaline soils) on the individual processes of C turnover. A recent study by Gregorich et al. (1991) in which they used ten soils of the same clay type but of ranging clay content, confirmed that biomass ^{14}C from ^{14}C -glucose decomposition, at least for 45 days incubation, was directly related to soil texture. However, unlike the data of Amato and Ladd (1992), which showed no trend in the amounts of non biomass ^{14}C retained relative to soil clay content, those of Gregorich et al. (1991) showed that non biomass ^{14}C concentrations were relatively high in soils of low clay content.

In the study in which calculations were made of gross first-order decay rates of ^{14}C , conclusions rested upon the assumption that the decomposer microflora in different soils utilized the broad range of substrates from the turnover processes with at least similar average efficiencies (Ladd et al., 1992). Investigations of substrate utilization efficiency according to soil and substrate properties, substrate location in micropores, and nutritional status during growth of the primary populations might aid in substantiating this assumption.

In the same study, the higher retention of total organic ^{14}C and the slower decay rate of biomass ^{14}C from glucose ^{14}C turnover in the Vertisol topsoil, compared with those in the Alfisol topsoil, appeared to be consistent with the hypothesis that the former soil offered greater opportunities for decomposer cells to be located at sites within micropores of neck sizes appropriate for cell protection, presumably from microfaunal predators (Ladd et al., 1992). The conditions of ^{14}C glucose addition favoured movement of substrate to pores of neck diameters nominally $< 6 \mu\text{m}$, and rapid metabolism of the added sub-

strate may have favoured growth of primary decomposers in pores of this size range. The high-clay Vertisol had a greater pore space in pores of neck diameters $< 6 \mu\text{m}$, than did the low-clay Alfisol (Jocteur-Monrozier et al., 1991).

Interpretation of results may need to be qualified by the following considerations.

(a) The Vertisol and the Alfisol had similar microporosities, i.e. the volume of pores with nominal neck diameters $< 6 \mu\text{m}$ and $> 0.2 \mu\text{m}$ (and below which little biomass ^{14}C would be expected to reside).

(b) Darbyshire (1975, 1976) has pointed out that microporosity per se does not reveal the pore network accessible to microfaunal predators; their movement will be influenced by the size of pores and the pore network containing adequate amounts of soil solution.

(c) The conditions of incubation provided for drainage of pores of diameters $> 6 \mu\text{m}$, and hence would have constrained predator movement and activity.

(d) Under the experimental conditions, differences in the ability of the primary decomposers to immobilize soil nitrate N in the presence of added ammonium N during active glucose metabolism resulted in the two soils forming microbial cells plus products of widely different C/N ratios, and possibly occupying disparate spatial niches within aggregates (Ladd et al., 1992).

DECOMPOSER ACTIVITY AND SUBSTRATE/ORGANISM LOCATION IN AGGREGATES

Substrate location and carbon turnover

Heijnen et al. (1990) reported increased survival rates of introduced bacteria (*Rhizobium* sp.) after their inoculation into soils at low rather than high initial soil water contents. The survival of the introduced cells was believed to be principally determined by their accessibility to predation by soil microfauna, and it was argued that amendment of the drier soils favoured movement by mass flow of the introduced cells to sites within pores of relatively small pore-neck diameters, thus affording them greater protection from attack by larger-sized predators.

The same rationale can be applied to the turnover of carbon from substrates introduced to different locations within the soil fabric. For many substrates, decomposition in situ of the applied substrate itself may be less influenced by its location within pores of different neck diameters than is the turnover of substrate-derived carbon, this being dependent upon the death and decay of cells of the primary decomposer populations, and which under suitable conditions will be strongly influenced by predator activity. Thus for substrates located in small protective microniches, turnover of substrate carbon would be slower, and biomass C accumulation would be higher than for

substrates decomposing in larger pores where decomposer cells were more vulnerable to attack by predators.

This hypothesis was tested by Killham et al. (1992) who applied ^{14}C -labelled glucose to a well-aggregated Vertisol at two matric potentials to introduce the substrate into soil pores of two size classes viz. $< 6\ \mu\text{m}$ and $6\text{--}30\ \mu\text{m}$ pore neck diameter. The labelled substrate was applied by nebulising solutions onto the surface of the thinly-spread soil which had been pre-equilibrated over a solution of saturated lithium chloride to ensure that all but the smallest pores were air-filled. The amounts of solution to be added were pre-determined from the moisture release characteristics of the soil. After a brief (3-day) incubation when all added glucose had been metabolized, incubation was continued at two matric potentials, thus enabling an evaluation of both substrate location and pore-water regime as controls of C turnover.

The results supported the hypothesis. During the first three days of incubation, added glucose essentially disappeared, and presumably as a result of its metabolism, contributed extensively to the rapid release of $^{14}\text{CO}_2$ during this period. There was no evidence to suggest that potential differences in microbiological populations or micro-environmental conditions (e.g. O_2 concentrations) at the two substrate locations substantially affected glucose decomposition itself.

However, thereafter when further metabolism of ^{14}C was comparatively slow and rested upon turnover of C through decomposer cells, rates were influenced by pore-size location of the added substrate and the soil water matric potential under which turnover took place (Table 1). Rates of decline of residual organic ^{14}C and biomass ^{14}C located in pores $6\text{--}30\ \mu\text{m}$ pore diameter were about 1.5 times greater than when located in pores of neck diameters up to $6\ \mu\text{m}$, but only when the pore water regime ensured that pores up to $30\ \mu\text{m}$ neck diameter were water-filled. When the pore-water regime was changed

TABLE 1

Effect of pore-size location of substrate and pore water regime on the net decay rates of biomass ^{14}C and total organic ^{14}C residual from ^{14}C -glucose metabolism

Substrate location (pore neck diam., μm)	Largest water-filled pore (pore neck diam., μm)	Net decay rate of residual $^{14}\text{C}^a$ (fraction of input ^{14}C day $^{-1}$)	
		Total organic ^{14}C	Biomass ^{14}C
<6	6	-0.009	-0.016
	30	-0.009	-0.016
6-30	6	-0.010	-0.015
	30	-0.015	-0.022

^aCalculated for the incubation period 3-23 days.

Data adapted from Killham et al. (1992)

such that only pores of neck diameters up to 6 μm were water-filled, turnover rates in the larger pores were reduced to approximately equal those in the smaller pores. The imposed soil water matric potentials did not influence turnover rates of organic ^{14}C located in the smaller pores. Although the experiment did not examine directly the involvement of predators in the turnover processes, the results of the treatments implied differential constraints on their access to primary decomposers.

In the above experiment use was made of a substrate glucose which was of low molecular weight, uncharged, highly soluble, and readily decomposable by the soil microflora. Under the experimental conditions used, the mass flow effects of transporting substrate to different pore locations in soils at different matric potentials and the rapid decomposition of substrate at both locations more than offset the equilibrating influences of substrate diffusion, resulting in differences in the location of the primary decomposer organisms. Further the soil water matric potentials established at the time of substrate application were chosen on the basis that the primary decomposers were bacteria, and that the neck size of pores in which they were located were critical for their survival.

Application of this approach to a range of soils, substrates and soil-water matric potentials, may help to establish the relative importance for carbon turnover of spatial compartmentalization of substrates and decomposers (primary and secondary); and so better to characterize soils in terms of their physical protective capacities to influence biological processes. Foster (1981b, 1985) using electron microscopy showed that many pores of sub-micron diameter in soils were filled with carbohydrate. Choice of neck sizes of pores in which added substrates are located, choice of substrate (molecular weight, solubility, chemical complexity) and accompanying nutrients (NH_4 , NO_3), may influence decomposition rates of the primary substrate as well as rates of turnover of substrate-derived carbon and nutrients.

For example, studies by Adu and Oades (1978a, b) followed the decomposition of ^{14}C -labelled glucose and starch after their incorporation into either macropores only, or micro- plus macropores of different sized soil aggregates. Prior to incubation the aggregates were sterilized then reinoculated with fresh soil suspension. From rates of evolution of $^{14}\text{CO}_2$ during a 24-day incubation, they concluded that starch but not glucose was protected from microbial attack when present in micropores of a fine sandy loam soil. Mechanical disruption of the incubated aggregates, or drying and rewetting of the aggregates induced greater flushes of $^{14}\text{CO}_2$ from the starch-amended soils than from those receiving glucose initially, reinforcing the notion of physical protective processes for substrate stability in soils.

Adu and Oades (1978b) also studied the effect of different cultures of fungi and bacteria added separately to sterilized samples of the amended aggre-

gates. Differences were observed depending on soil type, added organism type, and the pore size location of the substrate amendment.

Hattori (1988) examined the effects of soil moisture content prior to the addition of *E. coli* suspensions to sterilized aggregates, on the rates of oxidation of glucose added subsequently. Rates were greater when cells were introduced into both the outer and inner parts of the aggregates than when in the inner spaces only.

A further but related approach in characterizing soil physical properties and their importance for carbon and nutrient turnover might lie in the use of metabolic inhibitors or of biocides introduced into pores of different diameters of unamended soils, or perhaps in combination with amended soils in which substrates themselves are introduced into different locations within the aggregates. For example, Martin and Foster (1985) using electron microscopy, showed that micro-organisms located deep within pores or surrounded by gels and polysaccharide within the rhizosphere survived a 24 h exposure of soils to chloroform vapour, whereas cells located near the pore mouth or in spaces without mucigel were lysed.

Hattori (1988) summarized earlier studies which demonstrated that the numbers of Gram negative bacteria located internally within aggregates were less susceptible to sterilization by brief exposures of soil to the fumigant ethylene dibromide than were cells located in the outer parts of the aggregates. Similarly, enumeration of ammonium and nitrite oxidizers and heterotrophic bacteria of a soil treated with solutions of mercuric chloride demonstrated a greater susceptibility to this reagent by those cells located on the external surfaces of aggregates. Killham et al. (1992) showed that values for biomass ^{14}C from the metabolism of added glucose ^{14}C , were only slightly influenced by the initial pore-size location of the added glucose when assayed by the standard fumigation-incubation technique of Jenkinson and Powlson (1976). However, brief exposures (15–60 min) of the soils to chloroform indicated that the fumigant may have less-effectively accessed microbial biomass ^{14}C in smaller pores of the soil.

Substrate location and denitrification

The influences of pore-size location of substrates and microflora and microfauna extend beyond their effects on C and nutrient turnover in soils. For example, rates of denitrification are influenced by the spatial distribution of nitrate and carbon substrate at microsites within aggregates, and where potentially anaerobic conditions may develop through restrictions on O_2 diffusion (Sexstone et al., 1988). Air-filled pore space is an important determinant of the balance of aerobic and anaerobic microbial activities in soils (Linn and Doran, 1984). Denitrification in unamended, structurally intact, soil cores, placed in an aerobic atmosphere, was measureable even when the per-

centage of air-filled pore space exceeded 40%. Rates increased as the % air-filled pore space decreased (Sexstone et al., 1988). Denitrification potentially may cause large losses of N from soils at those water contents (> 60% water-holding capacity) which favour movement and activities of microfaunal predators (Darbyshire, 1976; Hattori, 1988). In soils with lower moisture levels (20–40% water-holding capacities), recoveries of NO_3 or NH_4 ^{15}N applied to soil crumbs generally exceed 90%, even in clay soils amended with glucose (Ladd et al., 1977, 1992). In these circumstances N immobilization is the dominant process.

There appears to be considerable scope for experimentation on the effects of pore location on the behaviour and activities of the soil native microflora, of micro-organisms introduced to soils, or of those generated in situ. However it is important to establish directly and independently, rather than by inference, the sites of activity in differently-treated soils, and to relate observed behavioural differences to sites within pores of ranging neck size.

CHARACTERIZATION OF ENZYMES IN SITU

Enzyme activity in soil was first demonstrated some 90 years ago, and today assays have been devised for a wide range of soil enzymes representing all enzyme classes. Old soils, even fossil soils, are enzymically active indicative of mechanisms for the protection of enzyme protein structure within the soil fabric. Nevertheless, direct evidence for the location of specific enzymes in soils is limited, and apportioning soil enzyme activity amongst that associated with live roots, the soil biota, cellular remnants (from roots and micro-organisms), faecal deposits (worm casts), and the soil mineral components has not been possible.

Electron microscopy and cytochemistry

Methods which have most successfully demonstrated at high resolution (<0.1 μm) the location of enzymes in soils, combine techniques of electron microscopy and cytochemistry.

In cytochemistry the target enzyme is supplied with its specific substrate, and one of the products of reaction is captured by a trapping agent to produce an insoluble, electron-dense precipitate. This accumulates in the immediate vicinity of the target enzyme so that the electron-dense marker is incorporated into the tissue at the site of the enzyme. Metals commonly used as trapping agents include lead or cerium (for phosphatases, aminotransferases), calcium (lipase), copper (cellulase, acyltransferases), iron/copper (dehydrogenases), barium (sulfatases), and osmium (peroxidases) (Lewis, 1977; Hayat, 1989). These methods are well established and of proven reliability in cells and tissues.

An advantage of these techniques is that they can be applied to whole aggregates so that numerous samples can be obtained for the detection of enzyme location at multiple sites. However, there may be problems of the accessibility of added substrate to target enzymes at internal microsites, and of the partitioning of reaction mixture components in their movement in soil to target enzyme locations. Further, comparisons of results from soils treated with the full complement of added substrate plus reagents, and from control soils treated with reagent but without substrate, are required to be carried out on separate aggregates.

Some of these methods have been applied to soils to locate acid phosphatase (Foster, 1985, 1988; Foster and Martin, 1981; Martin and Foster, 1985; Foster and Dormaar, 1992), alkaline phosphatase (Foster, 1985), adenosine triphosphatase (Foster, 1988), catalase (Heritage and Foster, 1984; Martin and Foster, 1985), peroxidase and succinic dehydrogenase (Foster and Martin, 1981) in roots, cellular debris and soil micro-organisms *in situ* in natural soils and model systems (Fig. 8).

In particular acid phosphatase has been demonstrated in the rhizodermal and root cap cells of roots, in soil fungi and bacteria, and in root surface mu-

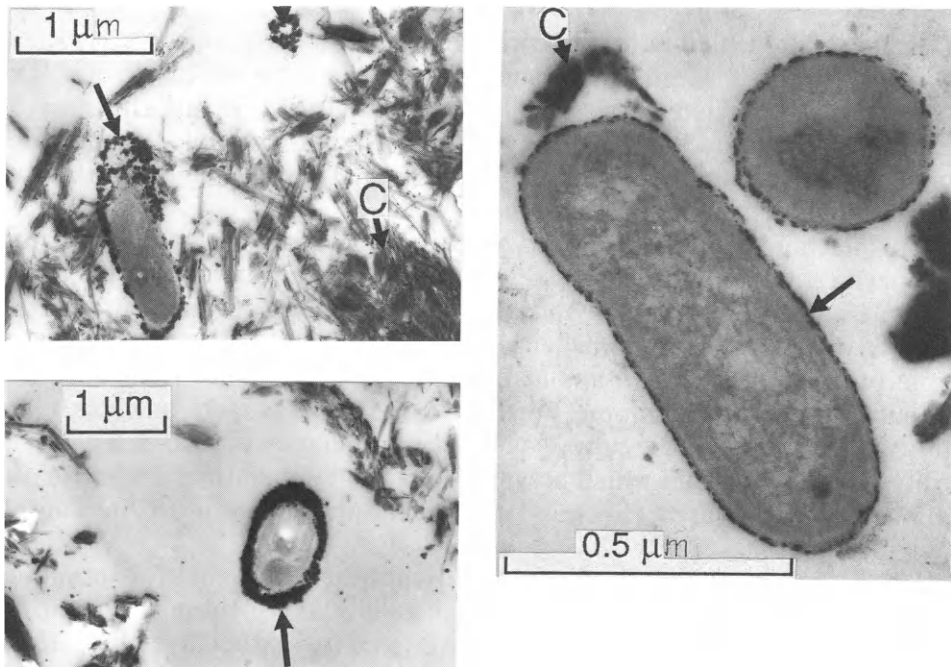


Fig. 8. In situ ultrastructural localization of enzymes associated with soil microorganisms. (top left) Acid phosphatase in intact bacteria. (bottom left) Acid phosphatase on remnants of cell membranes. (right) Catalase in a flooded rice rhizosphere. Refer to Fig. 1 for details.

cilage and on fragments of mucilage and microbial membranes deposited in the soil (Foster and Dormaar, 1992).

In the past, any enzyme activity associated with soil minerals and humified organic matter could not be unequivocally detected. Soil minerals have an intrinsically high electron density which masks any enzyme reaction product associated with them, and humified soil organic matter non-specifically adsorbs the heavy metal trapping agents necessary to confer electron density (and hence detectability) on the products of enzyme reactions. Electron probe microanalysis is overcoming some of these problems. The specificity of reactions can be tested, especially the testing of any non-specific staining of minerals etc. in controls where no substrate has been supplied, or where the enzyme has been inhibited. Results to date suggest that there is little non-specific attachment of metals to clays, and none to quartz, and there is little evidence for enzyme activity in clays. All detectable metal phosphates are associated with living cells.

Electron microscopy and immunocytochemistry

In this approach an antibody is raised against the target enzyme, labelled with an electron-dense marker (usually by conjugation with ferritin or colloidal gold), and applied to the sample such that the antibody attaches specifically to molecules of the target enzyme exposed on the sample surface. Sites of enzyme location are revealed by images of the electron-dense marker superimposed on the image of the sample.

This technique has been applied successfully in detecting sites of location of enzymes in tissues but not as yet in soils. The requirement that the target enzyme be isolated from soil and purified before preparation of its specific antibody presents a major challenge. The requirement too for ultrathin sections of specimen limits the application of the method to materials which can be cut with an ultramicrotome. Further, to maximize antigen-antibody reactivity, operations must be carried out at low temperatures (-20°C), and special embedding plastics which polymerize at low temperatures under UV radiation must be used. In soils this technique has resulted in uneven polymerization.

Gold labelling of an enzyme's antibody has the virtue that the technique can be made semi-quantitative, but in soils the gold colloid may become attached non specifically to humified organics to give false positives in controls. Also, gold markers are so small that high magnification electron microscopy must be used, resulting in high resolution but low and perhaps unrepresentative sample volumes.

CHARACTERISATION OF SUBSTRATES AND RESIDUES IN SITU

Electron optical techniques

Historically, the electron optical techniques most commonly used in soil organic matter studies have been transmission electron microscopy (TEM), scanning electron microscopy (SEM), and electron probe microanalysis (EPM). More recently laser microprobe mass analysis (LAMMA), secondary ion mass spectroscopy (SIMS), and electron energy loss spectroscopy (EELS) have had limited application in soil studies (Bisdorf, 1983).

Soil organic matter is generally composed of elements of low atomic number which are not directly detected by electron microscopy. Their presence may be indicated in TEM of soil sections as electron transparent spaces (e.g. surrounding roots or micro-organisms) in an otherwise dense mineral matrix, or as regions with low backscatter in SEM preparations. Materials with low backscatter and rich in S and P, as determined by EPM, have been identified as organic matter (McKeague et al., 1980).

SEM. For SEM, soil aggregates are fixed by aldehyde and osmium vapours, freeze-dried overnight, coated with carbon or gold, and mounted on carbon or aluminum stubs prior to their examination. In SEM soil organic matter appears as sheets of amorphous materials or fine fibrous deposits coating smooth and angular minerals.

TEM. For TEM, aggregates are enclosed in agar, fixed and subjected to histochemistry as required, dehydrated through alcohol or acetone, and embedded in resin. The resin-embedded soil blocks are then cut with an ultramicrotome using a diamond knife, and the ultrathin sections are either mounted on grids or subjected to further histochemistry (Foster and Martin, 1981).

In applying TEM methodology to ultrathin sections of biological tissues, the electron density and hence visibility of organic matter has been routinely enhanced by treatment with heavy metals, typically Os, Pb and U. These are unspecific stains which reveal almost all types of organic matter. Organic matter rich in polyphenols, S-containing and aromatic amino acids, and unsaturated lipids are particularly densely stained with Os and U, whereas carbohydrates are not. However, most organic materials, including carbohydrates, are stained by alkaline lead citrate. When applied to tissues the effects of these stains are unambiguous, but in soils heavy metals are known to complex with some minerals (e.g. oxides and hydroxides of iron), so their specificity for attachment to soil organic matter is in doubt.

TEM has been used to locate micro-organisms in microsites within and between soil aggregates, and in the rhizosphere (Foster, 1981a), and to inves-

tigate the decomposition of organic residues down to particles of submicron ($< 10 \mu\text{m}$) size (Foster, 1985).

Characterization of specific classes of organic residues

Humic materials. Humic materials stain densely with heavy metals. In plant fragments they are present in the middle lamellas and secondary cell walls of cells and tissues, and as granular deposits in cell vacuoles. In soils they are generally recognised morphologically when in particles $> 1 \mu\text{m}$, and by histochemical staining when in particles of submicron sizes. They occur as granules, sheets or fibres, depending on the pH and osmolarity of the surrounding soil solution (Chen and Schnitzer, 1989).

Carbohydrates. Of all the components of soil, carbohydrates have been shown to be the most closely correlated with aggregate stability. Soil carbohydrates are not stained by the conventional post-fixative OsO_4 so other techniques have been developed for particular types of carbohydrate. Substituted (e.g. acidic) carbohydrates, such as those in root and microbial gels, are stained with a ruthenium-red OsO_4 complex or with lanthanum hydroxide (Foster, 1981, a, b; Foster and Martin, 1981; Tiessen and Stewart, 1988). Neutral carbohydrates (e.g. structural carbohydrates of microbial and plant cell walls) are oxidized by periodate, followed by treatment with silver methenamine, or thiosemicarbazide/silver proteinate. Foster (1988) has dealt with the specificity of these stains, as applied to soil sections.

The ruthenium red and lanthanum stains have been introduced into natural soil fabrics to demonstrate both granular and fibrous microbial and root-derived gels. These gels have been observed to link micro-organisms to soil minerals and cellular debris to form microaggregates; and to link various soil components, including microaggregates, via fungal hyphae to form macroaggregates. Both these stains, when applied to soil sections, have shown that many of the finer micropores are occluded by carbohydrates derived from nearby roots or soil bacteria. These carbohydrates persist and bind aggregate components together, even when the bacteria themselves have autolysed and have vanished (Foster and Martin, 1981).

The periodate/silver methenamine and periodate/silver proteinate methods have likewise been used to investigate the decomposition of organic materials, mainly structural carbohydrates of plant and microbial cell walls. Micropores of submicron size have been shown to be occluded by such materials, which also bind together the mineral components of microaggregates (Foster, 1981b).

Lectins are natural compounds, usually derived from seeds, which bind specifically to particular monosaccharides. They can be labelled with a flu-

orescent tag to make them visible in the light microscope, and to colloidal gold to make them visible by electron optics, so that the sugars to which they attach can be specifically localised. Gold-labelled lectins have been widely applied to biological tissues to locate particular sugars on the surface of cells, but when applied to soils there are often non-specific reactions between the heavy metal tag and humic materials so that labelling is also obtained in control sections from which carbohydrates have been removed. We are not aware of the successful use of lectin histochemistry in the electron microscopy of natural soils, but it is a technique worth further investigation because of its narrow specificity.

Spectroscopic techniques

Spectroscopic techniques are often non-invasive, and therefore can provide detailed information on the nature of soil organic materials in situ. Two such techniques are nuclear magnetic resonance spectroscopy and Fourier transform infrared spectroscopy.

^{13}C NMR. With the advent of solid state ^{13}C NMR spectroscopy, detailed quantitative information on organic structures can now be obtained on whole soils and soil fractions (Wilson, 1987). The sensitivity of this technique for the ^{13}C nucleus is relatively low, and carbon levels $>2\%$ are generally required to obtain spectra with an acceptable signal-to-noise ratio (Skjemstad et al., 1986). Figure 9 shows the solid state ^{13}C NMR with cross polarization and magic angle spinning (CP/MAS) of a physically-separated fraction of

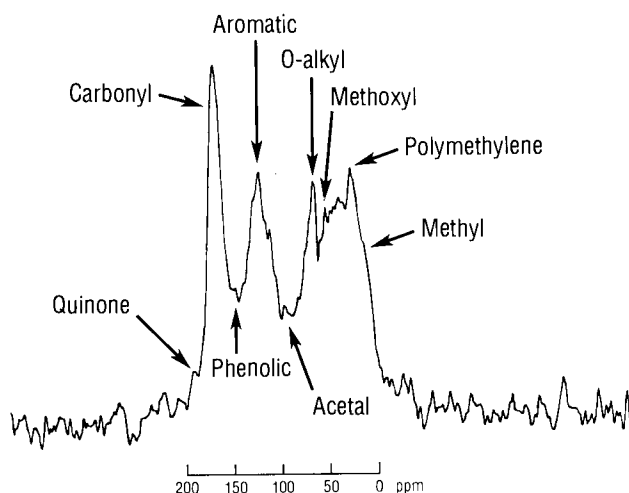


Fig. 9. Solid state CP/MAS ^{13}C NMR spectrum of organic matter from the Bh horizon of a podzol, showing the positions of various types of carbon nuclei.

the Bh horizon of a humus podzol, and indicates the assignments of the various resonance bands. Until recently, humified soil organic matter was thought to be dominated by aromatic rings but ^{13}C NMR studies have been able to demonstrate the presence of large quantities of alkyl (polymethylene) structures in the humus of many soils (Skjemstad et al., 1983). These substances are now known to persist (Skjemstad et al., 1986; Kögel-Knabner et al., 1991) and are most likely of microbial origin (Baldock et al., 1990; Capriel et al., 1990).

By performing experiments such as dipolar dephasing it is also possible to obtain additional information, such as the proximity of C nuclei to protons. This approach can be used, for example, to determine the degree of substitution of aromatic rings (Hatcher et al., 1989). As well, the molecular mobility of groups can be determined leading to a better understanding of their relationship with the surrounding environment (Wilson, 1987; Wilson et al., 1988).

Recently, Baldock et al. (1989) used ^{13}C enriched glucose in combination with ^{13}C NMR to determine the fate of this substrate during incubation. Since ^{13}C has a natural abundance of only about 1.1%, it is possible to add labelled substrates at reasonable concentrations. Using this novel approach, both the nature and the content of the metabolite carbon have been determined in a number of soil fractions. This technique shows considerable promise and will allow the fate of a number of carbon based substrates to be followed in situ.

^{31}P NMR. This nucleus has a 100% natural abundance, and the NMR technique for the ^{31}P nucleus is more sensitive than for ^{13}C . Solution studies of humic materials have shown the presence of a number of P-containing organic structures, including orthophosphate, mono and diesters, phosphonates, pyrophosphates and orthophosphate ions (Newman and Tate, 1980; Condon et al., 1985). Solid state ^{31}P NMR spectra have also been obtained on soil material (Williams et al., 1981) and have shown the presence of a mineral species similar to hydroxyapatite. Although ^{31}P NMR is relatively sensitive, the low concentration of P in organic materials means that the successful study by this technique of soil organic fractions other than humic acid-like substances is probably unlikely with present technology.

^{15}N NMR. This is another nucleus with a spin quantum number of 1/2, but with a natural abundance of only 0.366%. This makes detection difficult particularly considering the naturally low concentrations of N in soils. Benzing-Purdue et al. (1983) obtained useful spectra from ^{15}N -enriched synthetic melanoidins, and as with ^{13}C , the use of enriched substrates may provide a high enough concentration to allow useful spectra to be obtained with high field instruments (Wilson, 1987).

Infrared spectroscopy. Infrared spectroscopy (IR) has generally not been popular in soil organic matter studies except for the study of purified humic and fulvic acids incorporated into halide discs. With the advent of Fourier transform infrared (FTIR) spectrometers, considerably more potential now exists for whole soil studies. FTIR has two major advantages over dispersive techniques. Firstly, wavelength is extremely accurate due to the use of an internal laser, and secondly sensitivity is greatly enhanced. The former allows spectral subtractions to be made with a degree of confidence that spectral artifacts due to uncertainties in peak position have not been created. The latter means that low energy techniques such as diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), photoacoustic spectroscopy (PAS) and attenuated total reflection (ATR) spectroscopy are now possible on dilute samples.

Nguyen et al., (1991) demonstrated that useful DRIFT spectra could be obtained on powdered soil samples. Maintaining a fine and reproducible particle size in samples is important with this technique to minimize specular reflection. Samples are also often diluted with an IR-inert medium, such as KBr, to minimize distortions.

When studying soils and soil fractions by IR, the mineral components also absorb strongly and often correspond with organic absorption bands of interest. To overcome this difficulty, the technique of spectral subtraction can be employed. If organic matter can be destroyed without significantly affecting the background mineral spectrum, then subtracting the before and after spectra will lead to a spectrum of the lost organic fraction. In our laboratory, we are investigating two techniques by which these differences may be obtained. These are the use of O₂ with heat, and O₂ with high-energy, ultraviolet radiation. The former is convenient but often leads to mineralogical changes, whilst the latter must be achieved in suspension. Figure 10 shows (A) the original spectrum of a silt fraction, and the difference spectra obtained after (B) heating and (C) UV treatments. Note the appearance of mineral peaks (gibbsite) in the heated difference spectrum (B) in the 3500 and 1000 cm⁻¹ regions. Since the latter region coincides with the polysaccharide absorption region, it is impossible to determine the presence of this material by heating. The UV treated sample however does not suffer this limitation and therefore this approach can provide high quality spectra of organic fractions at concentrations far below those achievable by NMR, and in a fraction of the time.

Photoacoustic spectroscopy also shows considerable promise in soil organic matter studies. With this technique, the sample is placed within a sealed compartment connected to a supersensitive microphone. The IR radiation is passed through an IR transparent window onto the sample. The sample absorbs some of the radiation and emits it back into the gas in the chamber as heat. This thermal shock is detected by the microphone which acts as any other IR detector to give a weak IR spectrum. This technique is not very sensitive but the surface morphology of the sample does not lead to the spectral

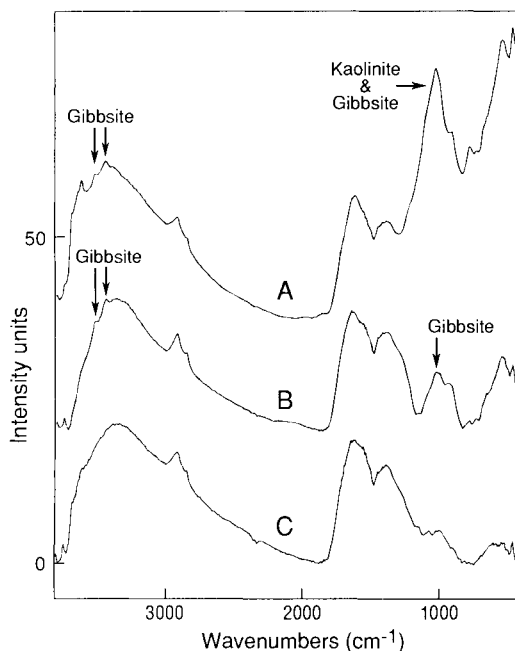


Fig. 10. IR spectrum of a silt fraction (*A*), and of a difference spectrum after heating (*B*) and UV treatment (*C*) of the fraction.

distortions seen with the DRIFTS technique. PAS appears to be ideal for the study of soil organic matter on surfaces with radiation only penetrating to a few microns. It is also possible to perform depth profiling experiments by changing some of the acquisition parameters.

Many spectral features in IR spectra can be identified such as O–H stretching of H bonded OH groups ($3400\text{--}3300\text{ cm}^{-1}$), C–H stretching of aromatic H (3050 cm^{-1}), alkyl C–H stretching ($3000\text{--}2800\text{ cm}^{-1}$), carboxyl–carboxylate C=O stretching ($1730, 1600, 1400, 1220\text{ cm}^{-1}$), amide I and II bands ($1650, 1550\text{ cm}^{-1}$), aromatic C=C ($1630\text{--}1580, 1500\text{ cm}^{-1}$), lignin ($1500, 1410, 1260\text{ cm}^{-1}$) and carbohydrates (1050 cm^{-1}). Quantification of these bands however is difficult although significant changes or trends in chemical composition can be readily identified. Because of the sensitivity and speed with which IR analysis can be performed with FTIR instruments, it is likely that a combination of IR and ^{13}C NMR spectroscopy will go a long way to unravelling the mysteries of the nature of soil organic matter.

The location of organic substrates in soil may be as significant a factor in their biological stability as their chemical composition. IR microscopy may provide a means of determining substrate distribution in a sample with a resolution of about $20\text{ }\mu\text{m}$.

This technique has been applied successfully to coals, plastics and many other materials, but not yet to soils.

FRACTIONATION OF SOIL AGGREGATES

Because spatial separation of substrate, enzyme and organism is believed to be an important determinant of the stability of organic matter in some soils, attempts have been made to characterize soil structure at the macro and microaggregate levels in terms which are relevant to biological activities. Such studies have often involved preliminary fractionation of soils by physical techniques to yield components of ranging particle size and density, and supported by subsequent chemical and biological analysis of the fractions.

Particle density fractionation

Fractions separated on the basis of particle density have yielded useful information on the extent of association of organic and inorganic materials in soils, as well as some chemical and mineralogical characterization of fraction components (Turchenek and Oades, 1979).

Generally soils are ground no finer than 0.2 mm thus ensuring that materials within microaggregates are not recovered as a light fraction. Fine grinding (Dalal and Mayer 1986), boiling (Monnier et al., 1962) and ultrasonification (Greenland and Ford, 1964) have been used in the past to maximize recovery.

Samples are suspended in solutions of known density and centrifuged to separate the materials with greater and lower density than that of the solution. Mixtures of organic solvents such as ethanol and bromoform or aqueous solutions of inorganic salts such as potassium iodide, zinc bromide and sodium polytungstate can be used. Sodium polytungstate is now widely used because it is nontoxic, can give solutions of s.g. as high as 3.1, and can be easily recovered for re-use.

The use of liquids or solutions with densities of $< 1.6 \text{ Mg m}^{-3}$ remove as a light fraction largely unaltered or partially degraded plant materials and charcoal (Skjemstad and Dalal, 1987; Skjemstad et al., 1990). With increasing density of the suspending medium, humified materials of increasing mineral content are isolated. The organics of these fractions are largely comprised of humic acids, which exist probably as organic aggregates weakly associated with clays (Skjemstad and Dalal, 1987).

The organic matter of light fractions ($< 1.6 \text{ Mg m}^{-3}$) is more prone to decomposition in disturbed soils e.g. in cultivated soils, than is the organic matter in the heavier fractions, and is considered an early indicator of the consequences of cultural practice (Ford and Greenland, 1968; Richter et al., 1975). Dalal and Mayer (1986) and Skjemstad and Dalal (1987) showed

that the rate of loss of carbon from a number of fractions separated from Vertisols decreased with increasing density and time of cultivation. The recovery of heavier fractions can be influenced by clay mineralogy (Spycher and Young, 1977) and therefore may be of limited value when comparing different soil types.

Particle size fractionation

Fractionation of soils based on the separation of components by particle size has been more widely used. Methods have extended from dry sieving, which may retain macroaggregates in the mm size range, to strong crushing of soils in mills and the use of chemical extractants to achieve complete soil dispersion.

Particle-size ranges frequently chosen for the fractionation of dispersed soils are (in μm), <0.04 , $0.04\text{--}0.2$, $0.2\text{--}2.0$ (clay-size); $2.0\text{--}5.0$, $5.0\text{--}20$, $20\text{--}50$ (silt-size); $50\text{--}250$, and >250 (sand-size). The amounts recovered in any particular fraction may depend greatly on the conditions of dispersion, e.g. pH, charge on the saturating cation, the total energy applied and the intensity of energy to which the particles are subjected (Edwards and Bremner, 1967; Theisen et al., 1968).

Biomass C recovery. The application of methods to achieve complete soil dispersion may give a more consistent particle size distribution over time than those applied for partial dispersion. However, the use in the former approach of higher energy inputs may in some soils cause massive losses of microbial biomass C and N (and ^{14}C and ^{15}N when combined in studies using isotope methodologies). Losses will vary with soils. For example, Ahmed and Oades (1984) used ultra sound to disperse a sandy-loam and a clay soil, and ATP analysis of extracts of soil fractions to determine biomass C. The dispersion and fractionation procedure resulted in 10% and 70% losses in biomass C in the respective soils. Amato and Ladd (1980) reported a 50% loss of biomass ^{14}C after complete dispersion of the same clay soil by brief (2 min) maceration of suspensions of the soil in water following extraction with 0.1M NaHCO_3 .

Such losses may seriously confound the interpretation of physical constraints on C and nutrient turnover, given the focal position conferred on biomass in the turnover processes and the likelihood that its location within aggregates may also influence its relative survival during soil dispersion. Further, cell destruction during soil dispersion may exacerbate the potential problems arising from differential redistribution of cells and cell products in particles during the total fractionation procedure. Metabolism of products from killed cells during the fractionation procedure would further distort the results of fraction analysis.

Partial dispersion of soils using gentler procedures such as that described by Brüchert (1979) may result in low (10%) or no loss of biomass C after fractionation. Indeed the amounts of biomass in the sum of the fractions may exceed that of the unfractionated soil, indicative of an enhancement of the efficiency of the assay when dealing with dispersed soil fractions.

For example, Jocteur-Monrozier et al. (1991) obtained recoveries of biomass C of 134% and 104% after gentle shaking with agate balls suspensions of an Alfisol and a Vertisol respectively. Recoveries of organic C and N after soil fractionation ranged from 85% to 100%. The Vertisol used was the same soil as previously used by Amato and Ladd (1980) and by Ahmed and Oades (1984), who reported large losses of biomass C using methods (blending, ultrasonics) for more complete soil dispersion. Subsequent studies by Jocteur-Monrozier, Ladd and Amato (unpublished data) in which the soils were amended and incubated with ^{14}C -glucose and ^{15}N - NH_4 , showed that recoveries of biomass ^{14}C after soil fractionation averaged 116% in the Vertisol, and 95% in the Alfisol. Recoveries of organic ^{14}C and total ^{15}N in fractions approached 100% in both soils.

Distribution of organic residues in size fractions. In these studies, native organic ^{12}C , organic ^{14}N and biomass ^{12}C were bimodally distributed in the size fractions, but with the greatest amounts by far of the organic residues located in the fine-silt size (20–2 μm) fraction of each soil, despite contrasting properties of this fraction from each soil (Ladd et al., 1990; Jocteur-Monrozier et al., 1991). The distributions of organic ^{14}C , immobilized ^{15}N and biomass ^{14}C from ^{14}C glucose/ ^{15}N ammonium metabolism in the Vertisol and the Alfisol (Fig. 11) were similar to those of the respective native organic residues (Ladd et al., 1990). Similar patterns of C and N distribution have been reported also for other soils, even in those subjected to energetic dispersion procedures leading to large losses of biomass C (e.g. Ahmed and Oades, 1984).

Changing distribution of organic residues with time. Procedures involving partial dispersion of soils and size fractionation may reveal with time shifts in particle-size weight distribution. Such shifts may have disproportionate effects on the distribution of fraction organic C, ^{14}C , N, ^{15}N etc., causing some difficulties in assessing the relative stabilities of the organic residues associated with the fractions. For example, Ladd, Van Gestel and Amato (unpubl. data) showed that a Vertisol, amended with ^{14}C -labelled plant residues and incubated in the field for six years, was less extensively dispersed by the Brückert technique than was the same amended soil incubated for seven weeks. Fraction weights in the 250–50 and 50–20 μm size ranges from the six-year incubated soil increased by 9% and 27% respectively, when compared with those from the seven-week incubated soil. Fraction weights in the 20–2 and 2–0.1 μm size ranges decreased by 7% and 18% respectively. As a result, the

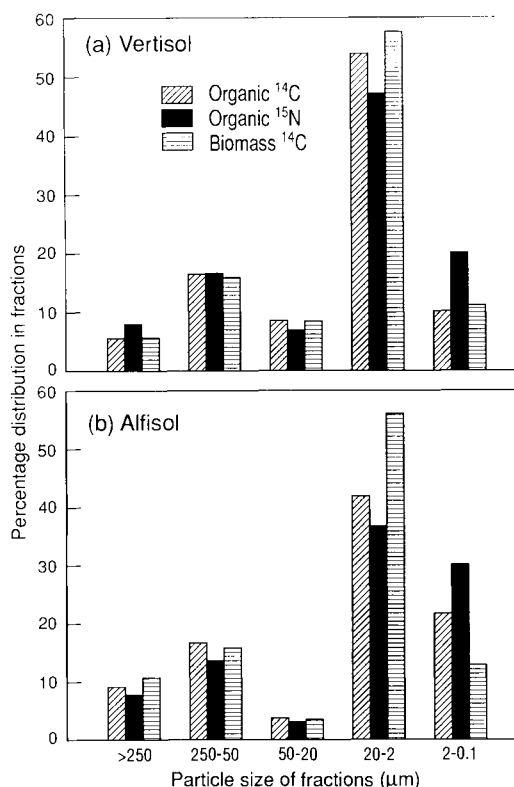


Fig. 11. Percentage distribution of organic ^{14}C , organic ^{15}N and biomass ^{14}C in particle size fractions of a Vertisol (a) and an Alfisol (b).

organic ^{12}C , organic ^{14}N and biomass ^{12}C contents of the 50–20 μm size fraction for example, increased by 50%, 54% and 67% respectively, in the six-year incubated soil when compared with those of this fraction from the seven-week incubated soil.

In the same experiment, interpretation of the relatively large decreases in total organic ^{14}C (72%), biomass ^{14}C (72%) and organic ^{15}N (49%) of unfractionated soil, in terms of the varying stabilities of residues in fractions, was more difficult. After correcting approximately for the effects of the differences in the ease of dispersion of the 7-week and 6-year incubated Vertisol, net changes in fraction organic ^{14}C and ^{15}N indicated that labelled residues of the coarse sand fraction were the least stable, and those of the 50–20 μm size fraction were the most stable. Nevertheless, in this study, as in others of its kind, net decreases will not have resulted from decay processes exclusively. Even after allowing for changed dispersive properties of the soil itself, changes with time in the type and location of decomposer populations, and in the ease

of dispersion of microbial colonies, may also lead to the transfer of organic ^{14}C and ^{15}N from one fraction to another.

Fractions have been characterized to varying degrees by the application of microbiological, biochemical, chemical, mineralogical, spectroscopic and microscopic techniques, some of which have been referred to above (sections IV and V).

A significant advance, in the context of modelling of carbon and nutrient turnover in soils, has been the development and widespread testing of fumigation/extraction techniques for the assay of microbial biomass, assays which can be used with advantage in conjunction with isotope methodologies (Hedley and Stewart, 1982; Brookes et al., 1985; Amato and Ladd, 1988; Tate et al., 1988). The fumigation/extraction assays perform well with soil slurries and are sufficiently sensitive, precise and simple to use for their easy application to soil particle size fractions.

UV photo-oxidation of aggregates. The development of a high-energy ultraviolet photo-oxidation technique may help to characterize both the nature of organic components and also their relationship(s) with inorganic particles, according to their location within aggregates. In this technique, soil fractions are suspended in water in a quartz tube and kept agitated by passing air through a capillary into the bottom of the tube. A high energy mercury vapour lamp (1000 W) is used to irradiate the sample, and in conjunction with the air causes rapid oxidation of the organic matter to more simple soluble molecules and ultimately to carbon dioxide. Thus far, the technique has been applied successfully to fractions of particle size $< 20 \mu\text{m}$.

Ideally its use selectively destroys organic matter on external surfaces of aggregates, leaving organic matter within aggregates intact. Preliminary data is encouraging, and coupled with spectroscopic techniques, is providing data on the nature of organic matter and the extent to which it is protected by virtue of its location within aggregates (Skjemstad, unpublished data). The value of this technique should be greatly enhanced when used in conjunction with isotopic techniques.

CHARACTERIZATION OF SITES OF ACTIVITY IN SITU

Studies with soil fractions have aided those with whole soils in defining the nature of substrates, enzymes and microorganisms within aggregates, and their relationships with soil minerals. The use of mildly-dispersed soils may reduce the potential for artifact error, whilst permitting comparisons of C and N behaviour in soils of ranging aggregate stabilities. However, whichever techniques are applied for dispersion and fractionation of soils, the results of fraction analysis are required to be interpreted in terms of the dynamics of turnover in whole soil aggregates, and this has proved to be difficult.

There are clear advantages when information which relates soil structure and biological activities can be gained without aggregate destruction. Grossbard (1969) used autoradiography to visually record the decomposition of ^{14}C -labelled plant materials either incorporated in soils or added to the soil surface. Decomposition was accompanied by dispersal of ^{14}C residues, and the appearance of labelled micro-organisms (especially fungi, during the initially rapid decay phase), and labelled faecal pellets, indicative of the participation of soil mesofauna.

The thickness of soil sections (ca. $40\text{ }\mu\text{m}$) prepared for light microscopy limits resolution to about $5\text{ }\mu\text{m}$. This is because unless they occur at the very surface of the section, micropores or objects of micron and sub-micron size (e.g. most soil bacteria) tend to be obscured by materials or objects lying above or below them, so they cannot be unambiguously identified.

Ultrastructural autoradiography has been widely used to locate sites of activity of specific enzymes in plant, animal and microbial cells, but not yet in whole soils (or soil fractions). The use of microautoradiography in soils would be invaluable, even to confirm sites within micropores of microbial activities which result from say, the introduction of substrates under different water regimes. Moreover, for modelling purposes, it would be advantageous to distinguish between active and repressed enzymes, and between metabolically active, inactive and dead bacteria, and between soil micro-organisms and their non-mobile residues such as cell walls and extracellular polysaccharides.

The principles and methodologies of macro- and micro-autoradiography have been described by L'Annunziata (1984). The methodology involved using defined tissues or cells has generally been as follows. Specific soluble substrates, suitably-labelled with radioisotope (tritium, ^{14}C , ^{32}P , ^{35}S , etc.), are added to and metabolized in selected samples. Specimens containing insoluble products of enzyme action are fixed, stained, and embedded in plastic by conventional techniques in TEM. Unreacted substrate and unfixed soluble products of reaction are washed out of the specimen during processing. Ultra thin sections are covered with a photographic emulsion and left for periods up to several months. When the emulsion is photographically developed and fixed, electron-dense particles of metallic silver are deposited at radiation-affected sites in the emulsion. In electron micrographs of the sections, images of the silver grains are superimposed on those of the specimens, enabling identification of the enzymically-active sites.

If a ^{14}C -labelled substrate were added to soil, all organisms in contact with and active against the substrate should become labelled, and therefore become detectable at low magnification in ultrathin sections of the aggregates. At higher magnifications, metabolically active hyphae and bacteria, individually active within colonies, could be distinguished from inactive cells. This technique may well provide direct visual evidence of the location of active micro-organisms in micropores in and between microaggregates according to

cell, substrate and soil properties, and the conditions influencing substrate movement by mass flow and diffusion.

By pulse labelling roots with D-[6-³H] glucose, Northcote and Picket-Heaps (1967) demonstrated the synthesis of a galactose-rich gel in the dicytosomes of root cap cells, the stages in its subsequent transfer to the plasmalemma in membrane-bound vesicles, and finally, its deposition on to the root surface by fusion of the vesicle membrane with the plasmalemma. Analogously, pulse labelling of soil aggregates may also well demonstrate the transfer of metabolized added substrate ¹⁴C from the cytoplasm of active microorganisms to their cell walls and exocellular polysaccharides, and to their predators and to higher orders of the food chain. Sites within aggregates, at which lytic and metabolic products are adsorbed, further transformed or are physically-protected, may also be indicated.

A major hurdle in the application of the method to soil may be one of sampling, since the volume of soil observed at the magnification required for adequate resolution of the electron-dense markers is so small (4 μm^3). All methods requiring ultramicrotomy of soil samples risk damage to diamond knives; and the need to embed samples in plastic risks artifact formation due to aggregate slaking, or to shrinkage during the dehydration procedures (Smart and Tovey, 1981). Most of these problems can be overcome or avoided (Foster and Martin, 1981).

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Effects of biocidal treatments on biological and nutritional properties of a mull-structured woodland soil

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ABSTRACT

Alpehi, J. and Scheu, S., 1993. Effects of biocidal treatments on biological and nutritional properties of a mull-structured woodland soil. In: L. Brussaard and M.J. Kooistra (Editors), *Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships*. Geoderma, 56: 435–448.

The effect of microwave radiation (MIW), gamma irradiation (GIR), autoclaving (ACL), methyl bromide fumigations (0.01 mol/l; MBR-1 and 0.04 mol/l; MBR-2), chloroform fumigation (CHL) and propylene oxide fumigation (POX) on soil respiration, N- and P-mineralization and pH of a rendzina soil was measured in a laboratory experiment over a period of 120 days.

Soil samples were taken from the Ah horizon of a beechwood mull rendzina on limestone (carbon content 8%, C/N ratio 14, pH 5.8). Control and treated soil samples were inoculated with a soil suspension (passed through a 45 μm sieve) and incubated in glass vessels at 20°C. CO₂ release was recorded and N_{min} content, P_{min} content and soil pH were determined.

Of the treatments, MIW, MBR-1 and CHL defaunated the soil but did not eliminate microbial populations, while soil sterilization was achieved by ACL, GIR, MBR-2 and POX.

In comparison to the control, CO₂ release of POX treated soil was increased strongly throughout the experiment. CO₂ release of the other treatments reached the control level after about four weeks of incubation. In MBR fumigated soil CO₂ production fell below the control level after 10 weeks indicating toxic effects of inorganic bromide residues in the soil.

The content of NH₄⁺-N was high and that of NO₃⁻-N was low in all treatments in comparison to the control. Nitrifying bacteria did not recover after being damaged by biocidal treatments. N_{min} content of POX treated soil was lowest, indicating immobilization of N for microbial growth. The phosphate content of treated soil samples generally exceeded that of the control soil throughout the experiment. Treatments increased soil pH by 0.2 to 0.7 units.

C-, N- and P-status of the soil was affected strongly by each of the treatments. For defaunation CHL and for sterilization GIR and ACL caused least side effects.

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INTRODUCTION

Soil processes are affected by a complex web of biological interactions (Hunt et al., 1987). To evaluate soil fauna–soil process causal relationships, systems of reduced complexity must be set up. Laboratory systems selectively inoculated with distinct faunal elements are considered to be the most useful approach (Anderson et al., 1979; Elliott et al., 1979).

Soil defaunation and sterilization procedures are basic requirements to set up reduced complexity laboratory systems. A variety of defaunation and sterilization procedures were investigated for their usefulness in these experiments (Speir et al., 1986; Wright and Coleman, 1988; Huhta et al., 1989). Each of the procedures used to eliminate metazoan fauna or microbial populations affects the physical, chemical, biological and nutritional properties of the soils too (Skipper and Westermann, 1973; Powlson and Jenkinson, 1976; Wolf et al., 1989). Therefore, the knowledge of side effects caused by defaunation and sterilization treatments is a basic requirement for planning, carrying out and interpreting laboratory experiments investigating interrelationships among soil organisms and their abiotic environment.

Side effects of defaunation and sterilization procedures have been investigated frequently. However, soils poor in C_{org} were used in most of the studies (Vela et al., 1976; Wolf et al., 1989) and only short-term side effects were taken into account (Powlson and Jenkinson, 1976). It is known that side effects of defaunation and sterilization procedures differ strongly in soils containing different amounts of organic matter (Eno and Popenoe, 1964). In addition, the content of C_{org} , and the soil structure, which is related to the soil humus content, may influence the efficiency of biocidal treatments. Therefore, the soil type has to be taken into consideration when evaluating the efficiency of biocidal treatments and the intensity of side effects.

The present study investigates side effects of biocidal treatments on a mull rendzina soil under beech (*Fagus sylvatica* L.) rich in C_{org} over a four month period. Seven different treatments [microwave radiation, gamma irradiation, autoclaving, methyl bromide fumigations] (0.01 mol/l and 0.04 mol/l), chloroform fumigation and propylene oxide fumigation were applied and soil respiration, N- and P- mineralization and pH of soil samples incubated for 120 days were investigated.

The aim of the study was to establish sterilization and defaunation procedures causing minor side effects, which enable the set up of experiments on soil biota/soil structure interrelationships in a mull-structured woodland soil rich in C_{org} closely resembling field conditions.

MATERIAL AND METHODS

Soil

In October 1990 soil samples (C_{org} 8%, C/N ratio 14, water content 44% of dry weight) were taken from the Ah horizon of a beechwood growing on a mull rendzina. The site is located on a limestone plateau east of Göttingen, Germany (Schaefer, 1990). Macrofauna and plant roots were removed by hand and the samples passed through a 4 mm sieve. Then, soil samples of 600 g fresh weight were placed in polyethylene bags. Sealed bags were stored at 4°C to await treatments. Control soil samples remained untreated at 4°C.

Biocidal treatments

Microwaving (MIW): 600 g of moist soil was evenly distributed on a glass plate and radiated in a commercial microwave oven (2450 MHz) at 700 W for 3 min.

Gamma irradiation (GIR): Sealed polyethylene bags (diameter 40 mm, height 200 mm) containing about 200 g of fresh soil were irradiated by a ^{60}Co source (400 TBq) for 33.7 h. Samples were rotated 180° in front of the source after 17 h. On average the samples received 0.10–0.12 MGy.

Autoclaving (ACL): Glass beakers containing about 200 g of fresh soil were autoclaved for 1 h at 120°C at each of three consecutive days.

Fumigations were performed in desiccators. Soil samples (600 g fresh weight each) were evenly distributed to a thickness of about 2 cm on a polyethylene sheet in the desiccator. *Methyl-bromide* was added either at a rate of 0.01 mol/l (MBR-1) or 0.04 mol/l (MBR-2) to evacuated desiccators, respectively. *Chloroform* (CHL) (stabilized with 60 ppm methyl butene; cf. Mueller et al., 1992) (40 ml) and *propylene oxide* (POX) (150 ml) were placed in beakers in the desiccator prior to evacuation. After fumigant application the desiccators were kept closed for 48 h. Then, biocides were removed by eight evacuation–ventilation cycles. Biocide-cleared samples were incubated at room temperature for 48 h. Then another fumigation cycle was applied followed by eight evacuation–ventilation cycles. Due to biocidal treatments the water content of the soil was reduced by 1–5%, except for MIW (13%) and ACL (8%). Subsamples were taken from treated and control soil to evaluate sterility and defaunation efficiency.

Sterility was tested by inoculating 4.5 ml of yeast tryptone soybean broth (YTSB) (Hensel et al., 1990) with 0.2 g fresh weight of soil. Tests were made in triplicate. After 7 days of incubation at 22°C non-sterile samples were recorded by visible microbial growth.

Nematodes were extracted from subsamples of 10 g fresh weight from each of the treatments using Baermann funnels in a modified O'Connor wet extraction apparatus (Heitkamp and Schauerermann, 1982).

A modified MPN method (Darbyshire et al., 1974; Wellner, unpubl.) was used to determine defaunation efficiency for Protozoa.

Incubation

Soil samples from each treatment equivalent to 30 g dry weight were placed in 1 liter glass vessels (12 replicates each) and inoculated with 2 ml of a soil solution prepared by shaking 30 g fresh soil with 300 ml H₂O and subsequent filtering through a 45 μ m sieve. Soil water content was adjusted to 60% of the dry weight corresponding to field capacity. The vessels were sealed and stored at 20°C in permanent darkness throughout the incubation period of 120 days.

CO₂ evolved in the vessels was trapped in alkali (5 ml 1N KOH) and determined titrimetrically (Macfadyen, 1970) at days 4, 10, 24, 38, 52, 66, 87, and 115. At days 0, 14, 28, 60 and 120 three replicates were destructively sampled from each of the treatments for mineral N, mineral P and pH determinations. Ammonium and nitrate were determined by extracting 20 g fresh weight of soil with 40 ml 1% KAl(SO₄)₂ and subsequent steam distillation and titration (Keeney and Nelson, 1982). Mineral P was extracted from 10 g fresh weight soil with 100 ml 0.5M NaHCO₃ and determined colorimetrically by the molybdophosphate complex-method (Olsen and Sommers, 1982). pH of soil samples was measured in the supernatant of a 0.01N CaCl₂ soil slurry one hour after mixing of soil and CaCl₂ solution (1:10).

Cumulative CO₂ evolution at day 115 and the last recordings of the other parameters measured were analysed by one-way analysis of variance. Differences between means were inspected using Tukey's honestly significant difference at the 0.05 level.

RESULTS

Sterility

Sterility of the soil was achieved by ACL, GIR, MBR-2 and POX treatments only, while MIW, MBR-1 and CHL defaunated the soil but did not eliminate microbial populations (Table 1). However, nematodes and protozoans responded differently to biocidal treatments. Few nematodes had survived in CHL and MBR-1 treated soils. Protozoa remained in MIW radiated samples.

CO₂ evolution

Soil respiration was strongly increased throughout the experiment in POX fumigated samples (Fig. 1). Respiration rates in POX treated soil increased rapidly reaching about 15 times the control level at day 24. At day 115 respiration rates in POX treated soil were still 3 times the control level.

TABLE 1

Sterilization and defaunation efficiency of different biocidal treatments for microorganisms, Protozoa and Nematoda

Treatment	Micro-organisms	Protozoa	Nematoda
CHL	+	—	+
MBR-1	+	—	+
MIW	+	+	—
MBR-2	—	—	—
ACL	—	—	—
GIR	—	—	—
POX	—	—	—

+: present, —: absent. CHL: chloroform fumigation, MBR-1: methyl-bromide fumigation (0.01 mol/l), MBR-2: methyl-bromide fumigation (0.04 mol/l), POX: propylene oxide fumigation, MIW: microwaving (3 min), ACL: autoclaving (3 × 1 h), GIR: gamma irradiation (0.10–0.12 MGy).

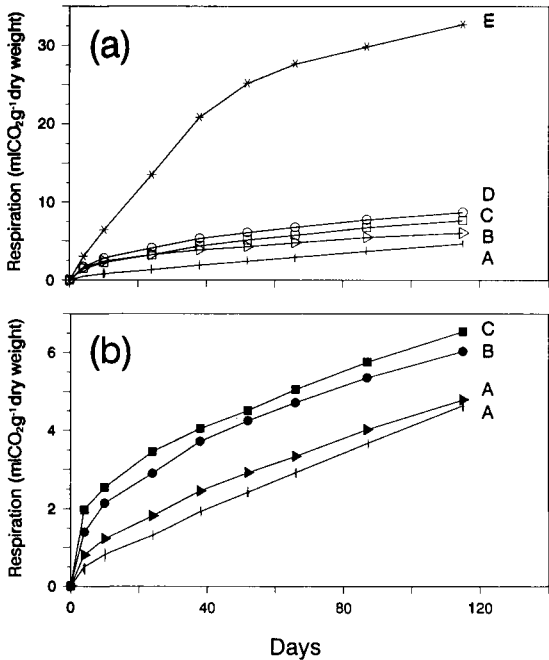


Fig. 1. Effect of different biocidal treatments on cumulative soil respiration of re-inoculated samples incubated in sealed 1 liter glass vessels. (a) Control (○) and treatments which achieved soil sterility: (□) ACL, (▴) GIR, (◊) MBR-2, (*) POX. (b) Control (○) and treatments which defaunated the soil only: (●) MIW, (▴) MBR-1, (■) CHL. Values of day 115 sharing the same letter are not significantly different (Tukey's HSD at the 0.05 level).

Respiration rates in ACL, MIW, MBR, GIR and CHL treated soil were also increased in comparison to the control but reached the control level later in the experiment.

Total CO₂ evolution of soil samples treated with methods which achieved sterility was in the range POX > ACL > GIR > MBR-2 > control (7.0:1.9:1.7:1.4:1) (Fig. 1a). In soil samples which had been defaunated only, total CO₂ evolution in CHL and MIW was similar and considerably higher than in MBR-1 and control samples (Fig. 1b). The comparatively low increase in CO₂ evolution in both MBR treatments was caused mainly by a reduction in CO₂ evolution rates during the second half of the experiment, which resulted in an approximation of cumulative CO₂ release in MBR treated and control samples (Fig. 1).

Mineral N

Ammonium was almost absent in the control soil throughout the experiment (Fig. 2). Except in POX fumigated soil the content of NH₄⁺-N increased strongly in each of the treated samples. Of the biocidal treatments which achieved sterility, ammonium mobilization was most pronounced in GIR and exceeded that of ACL and MBR-2 treated samples (Fig. 2). In gen-

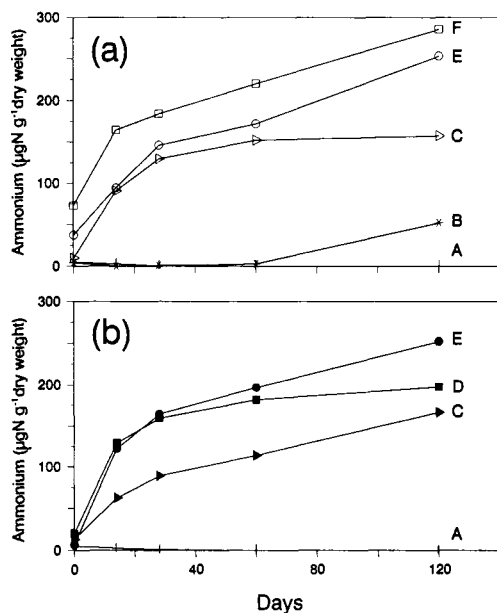


Fig. 2. Effect of different biocidal treatments on soil ammonium content of re-inoculated samples incubated in sealed 1 liter glass vessels. (a) Control and treatments which achieved soil sterility. (b) Control and treatments which defaunated the soil only. Symbols as in Fig. 1. Values of day 120 sharing the same letter are not significantly different (Tukey's HSD at the 0.05 level).

eral, biocidal treatments which defaunated the soil only caused a flush in NH_4^+ -N mobilization similar to that caused by sterilization procedures. Till day 28 the flush was most pronounced in GIR, lower in CHL, MIW, ACL and MBR-2 treated samples and least pronounced in MBR-1 fumigated soil. During the second half of the experiment NH_4^+ -N mineralization was high in GIR, ACL, MIW, MBR-1 and POX treated soil and low in CHL and MBR-2 fumigated samples.

The nitrate content increased almost continuously during the experiment in the control soil (Fig. 3). In contrast, in biocidal treated soil the nitrate content remained almost constant throughout the experiment except in POX fumigated samples. POX fumigation resulted in almost total immobilization of nitrate within the first 14 days of the experiment. Little NO_3^- -N mobilization may have occurred in MBR-2 and CHL fumigated soil during the second half of the experiment.

Ammonium and nitrate mobilization and immobilization resulted in different mineral nitrogen status in treated soil samples at day 120 (Fig. 4). Except for POX, sterilization procedures caused an increase in mineral N in comparison to the control by factors of 3.1, 2.7, 2.0 for GIR, ACL, MBR-2

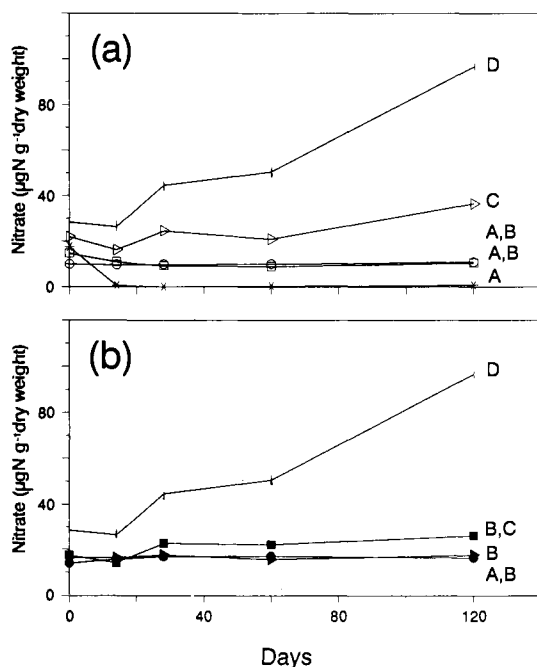


Fig. 3. Effect of different biocidal treatments on soil nitrate content of re-inoculated samples incubated in sealed 1 liter glass vessels. (a) Control and treatments which achieved soil sterility. (b) Control and treatments which defaunated the soil only. Symbols as in Fig. 1. Values of day 120 sharing the same letter are not significantly different (Tukey's HSD at the 0.05 level).

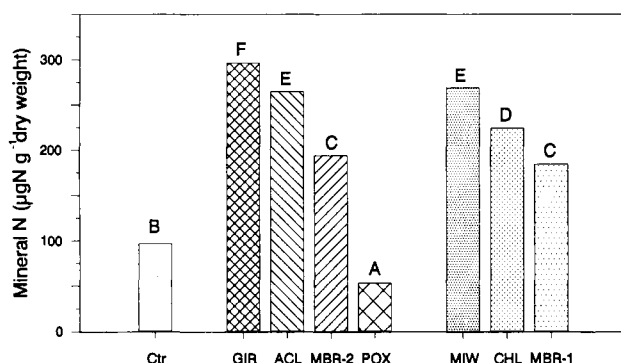


Fig. 4. Content of mineral N in control and biocidal treated and re-inoculated soil samples after 120 days of incubation. *Ctr*: control, *GIR*: gamma irradiation, *ACL*: autoclave, *MBR-2*: methyl bromide (0.04 mol/l), *POX*: propylene oxide, *MIW*: microwave, *CHL*: chloroform, *MBR-1*: methyl bromide (0.01 mol/l). Columns sharing the same letter are not significantly different (Tukey's HSD at the 0.05 level).

treatments respectively. In POX fumigated soil mineral N content was 45% below the control level. Biocidal treatments which defaunated the soil only resulted in an increase in mineral N content in comparison to the control by factors of 2.8, 2.3, 1.9 for MIW, CHL, MBR-1 respectively at day 120.

Mineral P

The content of mineral P in the control soil was low and remained almost constant throughout the experiment (Fig. 5). Each of the biocidal treatments caused a strong increase in the mineral P content of the soil. The amount of mineral P in soil depended on fumigation treatment and varied with time. A strong increase in P mobilization occurred in POX, MBR-2 and CHL fumigated soil within the first 14 days of incubation. After this initial flush, P was immobilized in these biocidal treated soils. At day 120 the mineral P content in soil samples which had been sterilized was increased in comparison to the control by factors of 4.6, 3.1, 2.9 and 2.0 for GIR, ACL, MBR-2 and POX respectively. Biocidal treatments which defaunated the soil only resulted in a similar mineral P content about three times the control level at the last recording.

Soil pH

The pH in the control soil increased from 5.8 to 6.5 during the first 14 days of incubation and then decreased to 5.9 at the end of the experiment (Fig. 6). In general, the pH in biocidal treated soil samples exceeded the control level considerably. The differences were most pronounced at the end of the exper-

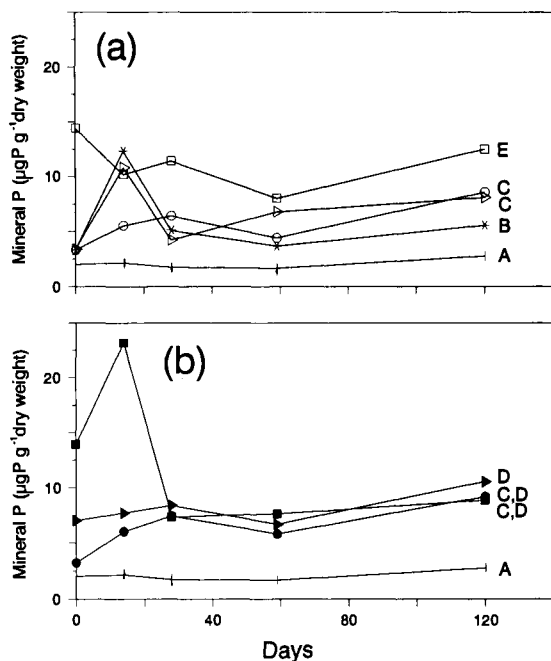


Fig. 5. Effect of different biocidal treatments on soil phosphate content of re-inoculated samples incubated in sealed 1 liter glass vessels. (a) Control and treatments which achieved soil sterility. (b) Control and treatments which defaunated the soil only. Symbols as in Fig. 1. Values of day 120 sharing the same letter are not significantly different (Tukey's HSD at the 0.05 level).

iment when the pH of POX, MBR-2 and CHL fumigated samples was 1.0–1.2 and that of ACL, GIR, MIW and MBR-1 treated samples 0.5–0.6 pH units above the control level.

DISCUSSION AND CONCLUSIONS

Soil microorganisms and certain faunal groups are known to survive severe physical and chemical environmental alterations (Jenkinson, 1966; Powlson and Jenkinson, 1976; Huhta et al., 1989).

Of the biocidal treatments used in the present study, only ACL, GIR, MBR-2 and POX achieved soil sterility. CHL and MBR-1 treatments were found to effectively defaunate protozoans. However, although being effective for Protozoa, defaunation by CHL and MBR-1 treatments was incomplete for nematodes, whereas MIW resulted in complete nematode elimination. It is known, that certain nematode groups withstand adverse conditions in anhydrobiotic forms and therefore are difficult to eliminate (Freckman, 1978; Huhta et al., 1989). Additional experiments showed that complete nematode removal can be achieved by a third application of MBR-1 and CHL fumiga-

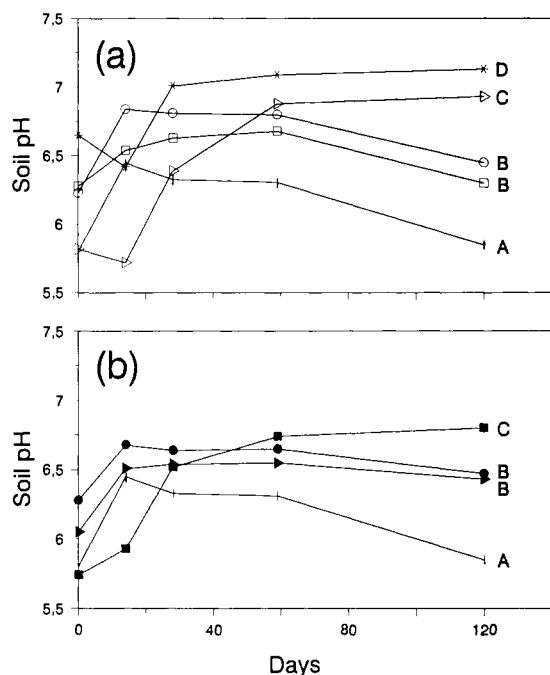


Fig. 6. Effect of different biocidal treatments on soil pH of re-inoculated samples incubated in sealed 1 liter glass vessels. (a) Control and treatments which achieved soil sterility. (b) Control and treatments which defaunated the soil only. Symbols as in Fig. 1. Values of day 120 sharing the same letter are not significantly different (Tukey's HSD at the 0.05 level).

tion following two days of incubation at room temperature (J. Alpheï, unpubl. data). The efficiency of MIW defaunation for Protozoa was not entirely successful. Flagellates and amoebae but no ciliates were still present in samples radiated for 3 min. Huhta et al. (1989) also found protozoans to survive two consecutive 380 W microwave treatments for 3 min. Presumably, defaunation of Protozoa can be achieved only by multiple MIW applications for longer periods of time. Despite the fact that no other faunal groups were investigated in the present study, it is assumed that defaunation was entirely successful if protozoans and nematodes had been effectively eliminated.

Each of the biocidal treatments, which achieved soil sterility caused strong side effects on soil biological and nutritional properties. In general, the total soil respiration was increased by these treatments. In addition, the $\text{NH}_4^+ - \text{N}$ content increased continuously except for POX treated soil throughout the experiment. In contrast to the control, the nitrate content remained on an almost constant low level in soil of each biocidal treatment except POX. The mineral P-content in soil was generally increased strongly due to biocidal treatments throughout the experiment.

Of the treatments which achieved sterility POX fumigation caused the most

striking effects on soil nutritional properties. Toxic effects of POX fumigation are known to be caused by esterification of carboxyl and phenolic hydroxyl groups in organic matter proteins (Fraenkel-Conrat, 1944; Wolf et al., 1989). Esterified POX residues were shown to cause reduced germination and growth of plants even after detoxication (Skipper and Westermann, 1973). Chemical reactions of POX with soil organic components also increases the pH in soil considerably by neutralization of labile protons of inorganic and organic soil fractions (Fraenkel-Conrat, 1944; Bartlett and Zelazny, 1967; Wolf et al., 1989).

POX treatment resulted in a dramatic increase in CO₂ evolution indicating high microbial respiration and growth. Presumably, the microflora used not only C_{org} solubilized from cell material by the fumigation process but also exploited the C-pool of POX residues (Anderson et al., 1978) in soil. Increased C utilization was accompanied by rapid N immobilization indicating microbial N allocation for growth. Presumably due to depletion of POX residues after about 60 days, rates of CO₂ release from POX fumigated soil had declined to control level.

In contrast to POX, CO₂ evolution was low in MBR fumigated samples. After the initial flush, respiration rates fell below the control level later in the experiment. MBR decomposes through chemical reaction with soil organic matter to methanol and inorganic bromide, which persists in soil and may cause toxic effects on microorganisms and plants (Maw and Kempton, 1973; Thompson, 1990). In addition, MBR acts as an enzyme inhibitor blocking active sites of enzymes by reaction with NH₂ and SH groups (Powlson and Jenkinson, 1976). The drop in CO₂ evolution in MBR treatments indicates long-term toxic effects of bromide residues on the soil microflora.

Both fumigants (POX and MBR-2) used to sterilize soil left residues in the soil, which strongly affected the carbon and nutrient turnover. Therefore, methyl-bromide and propylene oxide are assumed to be less useful fumigants for studies on energy and nutrient dynamics in soil.

Autoclaving and gamma irradiation are frequently used procedures to sterilize soil samples. Usually gamma irradiation is preferred to autoclaving, because strong side effects of autoclaving have been described (e.g. Russel and Hutchinson, 1909; Powlson and Jenkinson, 1976). In the present study, however, side effects of gamma irradiation on soil nutritional properties exceeded that of autoclaving. In contrast, the initial flush in CO₂ production was higher in autoclaved than in gamma irradiated soil. Both treatments have been shown to kill not only soil organisms but also disrupt soil organic material (Eno and Popenoe, 1964; Coleman and Macfadyen, 1966; Jenkinson, 1966).

Despite the strong side effects of autoclaving and gamma irradiation, mull-structured beechwood soil sterilized by these procedures should be preferred to chemically sterilized soil in laboratory incubations, because of long-term effects of residues of chemical treatments.

Compared to micro-organisms soil animals are more susceptible to biocidal treatments. Hence, defaunation procedures are expected to cause less side effects than soil sterilization. In the present study, microwave radiation, which effectively killed nematodes but not Protozoa, caused a flush in CO₂ evolution similar to that caused by autoclaving and gamma irradiation. In addition, mobilization of N in MIW treated soil was almost identical to that in autoclaved and gamma irradiated samples. Killing of soil organisms by microwaving and autoclaving is caused by soil heating. Hence, similar side effects of both treatments can be expected. Speir et al. (1986) found that 95% of the microbial biomass is killed by 90 s of microwave radiation. The defaunation efficiency depended on the soil water content and the amount of soil radiated (Vela et al., 1976). In addition to changes in soil nutritional properties, microwaving was shown to affect physical properties of the soil such as water holding capacity (Huhta et al., 1989).

Protozoa and nematodes were found to be killed by two or three fumigation–detoxication cycles with chloroform. CHL fumigation also caused a flush in microbial respiration and increased the content of mineral N and P. The flush in CO₂ production following chloroform fumigation is known to be caused by decomposition of fumigated organisms in the soil (Jenkinson, 1976). In addition, in contrast to fumigation with POX and MBR, chloroform can be removed from the soil effectively and soil humus material is only slightly affected. Hence, of the biocidal treatments studied, chloroform fumigation is assumed to be the most useful procedure to obtain defaunated soil for laboratory incubations using mull soils rich in C_{org}.

In each of the biocidal treatments except POX the ammonium content increased continuously, whereas the amount of nitrate remained almost constant. In contrast to the control soil no nitrification occurred. Obviously, nitrifying bacteria, which were damaged by biocidal treatments, did not recover during the experiment. Susceptibility of nitrifying bacteria to biocidal treatments is well known (e.g. Russel and Hutchinson, 1909; Thompson, 1990). The observed increase in soil pH in biocidal treated samples is assumed to be related to the lack of nitrification. Nitrification is known to be accompanied by proton production (Ulrich, 1981). Hence, the effect of biocidal treatments on soil pH can be assessed only against the background of nitrogen mineralization processes.

One of the most serious side effects of sterilization and defaunation of woodland mull soils is the strong increase in nutrients caused directly by fumigant application and indirectly due to a prolonged increase in nutrient mineralization in fumigated samples. Woodland mull soils are characterized by low nutrient availability to the soil microflora and the microflora is limited by nutrients or has a high demand of nutrients, particularly N and P (Scheu, 1990; Scheu, 1993). To obtain sterilized or defaunated mull soil rich in C_{org}

closely resembling field conditions, the treated soil samples should therefore be thoroughly leached before experiments are set up.

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Methods for physical separation and characterization of soil organic matter fractions

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ABSTRACT

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Turnover of soil organic matter (SOM) is coupled to the cycling of nutrients in soil through the activity of soil microorganisms. Biological availability of organic substrate in soil is related to the chemical quality of the organic material and to its degree of physical protection. SOM fractions can provide information on the turnover of organic matter (OM), provided the fractions can be related to functional or structural components in soil. Ultrasonication is commonly used to disrupt the soil structure prior to physical fractionation according to particle size, but may cause redistribution of OM among size fractions. The presence of mineral particles in size fractions can complicate estimations of OM turnover time within the fractions. Densimetric separation allows one to physically separate OM found within a specific size class from the heavier-density mineral particles. Nutrient contents and mineralization potential were determined for discrete size/density OM fractions isolated from within the macroaggregate structure of cultivated grassland soils. Eighteen percent of the total soil C and 25% of the total soil N in no-till soil was associated with fine-silt size particles having a density of 2.07–2.21 g/cm³ isolated from inside macroaggregates (enriched labile fraction or ELF). The amount of C and N sequestered in the ELF fraction decreased as the intensity of tillage increased. The specific rate of mineralization (μg net mineral N/ μg total N in the fraction) for macroaggregate-derived ELF was not different for the three tillage treatments but was greater than for intact macroaggregates. The methods described here have improved our ability to quantitatively estimate SOM fractions, which in turn has increased our understanding of SOM dynamics in cultivated grassland systems.

INTRODUCTION

The formation and turnover of soil organic matter (SOM), primarily driven by the activity of the soil microbial biomass, are closely coupled to the cycling of nutrients in the soil. The biological availability of organic substrate in soil is related to the chemical quality of the organic material and to its degree of physical occlusion, which can determine the accessibility of the ma-

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terial to soil microorganisms (Elliott and Coleman, 1988). information on the turnover of soil organic matter can be obtained by using soil fractions, provided the isolated fractions can be related to structural or functional components in soil, and thereby, to biological turnover (Christensen, 1987).

Physical fractionation of soil according to particle size has been used extensively to study soil organic matter (Edwards and Bremner, 1967b; Turchenek and Oades, 1979; Anderson et al., 1981; Tiessen and Stewart, 1983; Christensen, 1985; Balesdent et al., 1988; Jocteur Monrozier et al., 1991) and the methods have proven to be useful in revealing differences in the structural and dynamic properties of organic matter (OM) from different soils and particle size fractions (Christensen, 1987). Ultrasonication is the most common method used to obtain a high degree of disruption of the soil structure prior to physical fractionation (Anderson et al., 1981; Christensen, 1985). The greatest potential problem associated with the use of sonication prior to physical fractionation is the redistribution of organic matter among fractions. Differences in soil organic matter distributions may reflect inherent soil differences, but they may also arise as a result of differences in methodology (Christensen, 1985). For example, increasing the intensity of sonication can result in distribution of more organic material into finer soil fractions compared with a lower intensity of sonication energy (Elliott and Cambardella, 1991). The degree of soil disruption produced by a given ultrasonic energy level is often standardized by comparing the particle size distributions to those obtained from more classical chemical dispersion methods (Edwards and Bremner, 1967b). Since soil organic matter usually composes a relatively small fraction of the total soil weight, changes in organic matter distribution among fractions will not significantly affect the particle size distribution, while it may affect the concentration of organic matter within the particle size fractions (Elliott and Cambardella, 1991). The presence of mineral particles in size fractions can complicate estimations of organic matter turnover time within the fractions. Densimetric separation allows one to physically separate organic matter found within a specific size class from the heavier-density mineral particles.

The objective of this study was to develop a method to isolate and characterize soil organic matter fractions that were originally occluded within the aggregate structure of the soil and to utilize this information to increase our current level of understanding about how the aggregate structure controls the turnover of soil organic matter.

LABORATORY METHODS

Water-stable aggregates

Soil subsamples (100 g) were passed sequentially through a series of three sieve sizes to obtain four aggregate size fractions: (1) $> 2000 \mu\text{m}$ (large ma-

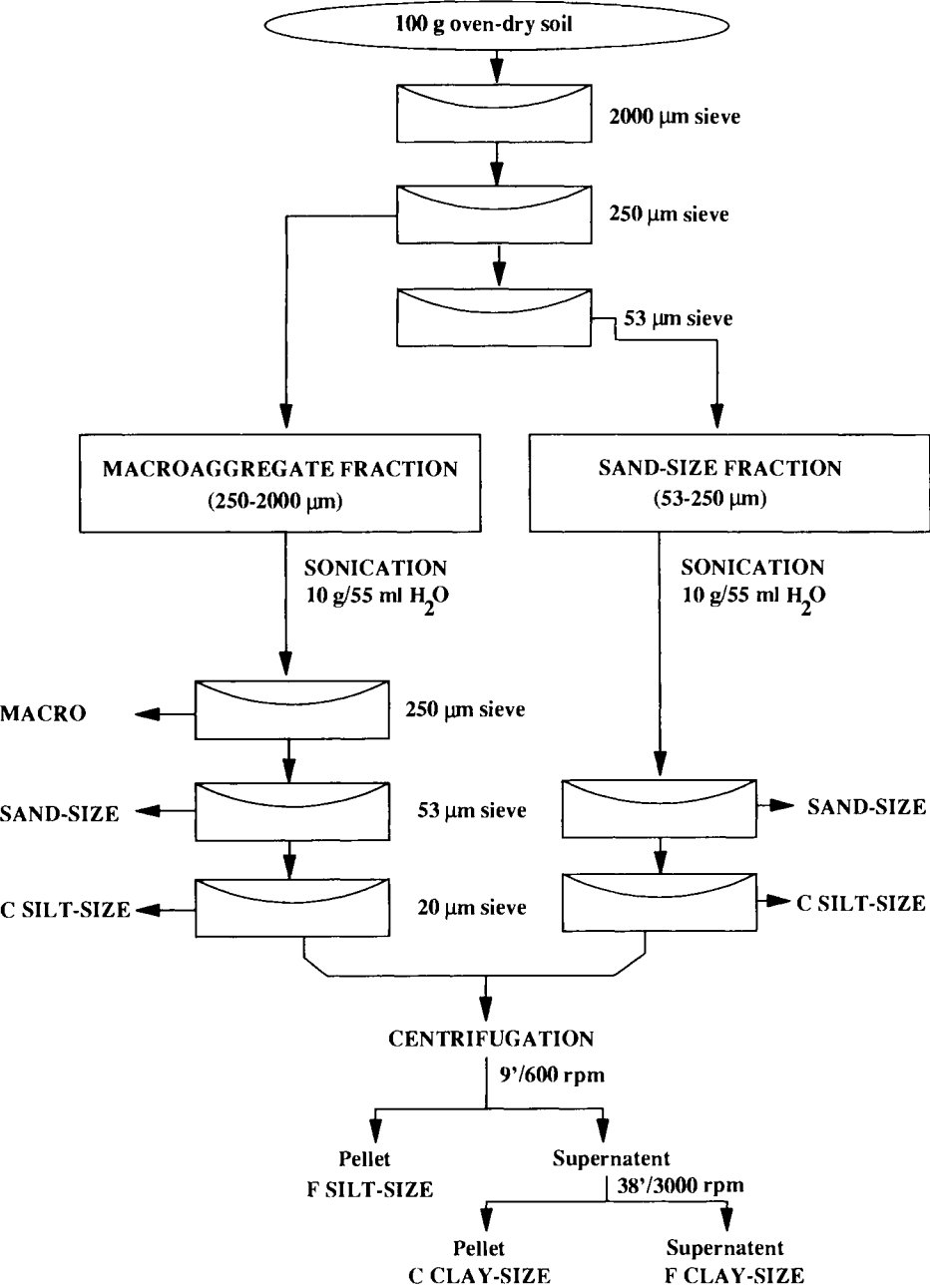


Fig. 1. Size fractionation sequence. All size fractions were dried overnight at 50°C.

croaggregates) (2) 250–2000 μm (small macroaggregates) (3) 53–250 μm (microaggregates) and (4) < 53 μm (Fig. 1). Before wet sieving, the samples were capillary-wetted overnight at 4°C (Hofman and De Leenheer, 1975). Since the moisture content of the soil at the time of wet sieving affects the aggregate size distribution (Kemper and Rosenau, 1986), the capillary-wetted soils were consistently brought up to field capacity plus 5%, the moisture content at which maximum aggregate stability is attained for these soils. The pre-wetted soil samples were suspended in water at room temperature on the largest sieve for five minutes before sieving. Floatable particulate organic matter was removed from the surface of the water during this time period with a polypropylene sponge and discarded. Aggregate separation was accomplished by moving the sieve 3 cm vertically 50 times over a period of 2 minutes, being careful to break the surface of the water with each stroke. Material remaining on the sieve was backwashed into a round aluminum cake pan and dried at 50°C overnight in a forced-air oven. Soil that passed through the sieve and into the water was poured through the next finer sieve size and the process repeated. The smallest fraction (silt plus clay) was centrifuged 10 min at 600 rpm and the pellet backwashed to an aluminum cake pan and dried overnight at 50°C (Elliott, 1986). The dried aggregate size fractions were weighed and stored in wide-mouth snap cap vials at room temperature.

Prior to chemical analysis, plant residue and roots that were larger than about 1 mm in length were removed by hand with a forceps from subsamples of the aggregate size fractions. The aggregates were ground in a mortar and pestle and subsampled to determine total organic C (Snyder and Trofymow, 1984) and total Kjeldahl nitrogen (Nelson and Sommers, 1980) by wet oxidation.

Intra-aggregate organic matter fractions

Aggregates were disrupted by sonication using a probe-type ultrasonic unit (Ultrasonics Heat Systems Model W-375). Ultrasonic vibration imparts vibrational energy to the soil solution causing cavitation, the formation of microscopic bubbles. In soil suspension, it is the collapse of these bubbles that produces pressure waves of sufficient mechanical energy to disrupt the bonds responsible for aggregation (Gregorich et al., 1988). The probe output power was calibrated by measuring the heating and cooling temperature changes produced by sonicating a known mass of water for a specific time (North, 1976). The energy output was determined to be 6.87 ± 0.09 J/s when the sonicator was set at 15% of maximum output power. The sonication time was adjusted to produce the level of sonication energy used in our experiments (1236 J). This energy level resulted in a complete breakdown of small macroaggregates (250–2000 μm) into sand and silt-size microaggregates.

Aggregate disruption was accomplished by suspending 10 g of dried aggre-

gate soil in 60 ml of water at room temperature and immersing the ultrasonic probe to a depth of 3–5 mm into the soil suspension. The disrupted soil suspensions were passed through a series of sieves in order to separate size fractions that were contained within the structure of the original aggregates. Four size fractions were isolated from the sonicated macroaggregates: (1) $> 250 \mu\text{m}$, (2) $53\text{--}250 \mu\text{m}$, (3) $20\text{--}53 \mu\text{m}$ and (4) $< 20 \mu\text{m}$ (Fig. 1). Fine-silt size and clay-size material were isolated by sequential centrifugation. All size fractions were dried overnight at 55°C in a forced-air oven. The $< 20 \mu\text{m}$ size fraction was determined to be 96% fine-silt size ($2\text{--}20 \mu\text{m}$), 3% coarse-clay size ($0.2\text{--}2 \mu\text{m}$) and 1% fine-clay size ($< 0.2 \mu\text{m}$).

Density separations of aggregate-derived size/density fractions were carried out by suspending 0.75 g subsamples of size fraction in 15 ml of sodium polytungstate (Oades, 1988) adjusted to a density of 1.85, 2.07, or 2.22 g cm^{-3} (Fig. 2). The suspended soil was evacuated for 10 min under a 186 kPa vacuum to remove entrapped air from the soil pore space. Organic material

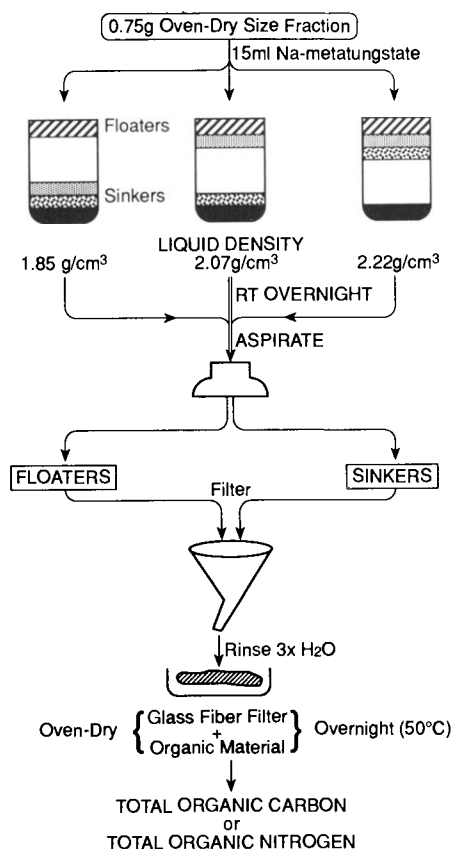


Fig. 2. Densiometric fractionation sequence.

that was equal to or lighter than the liquid in which it was suspended floated to the top after sitting overnight at room temperature. Organic matter floating on the surface of all density suspensions was removed by aspiration, washed several times with deionized water, trapped on a pre-ashed glass fiber filter and dried overnight at 55°C. The organic matter that sank in the 2.22 g cm⁻³ density liquid was also isolated in the same fashion. Total organic C (Snyder and Trofymow, 1984) and N (Nelson and Sommers, 1980) were estimated by wet oxidation of the filter plus adhering organic matter. Sodium polytungstate did not interfere with the digestion procedures. Total organic carbon or nitrogen was expressed as the total amount of carbon or nitrogen in the size/density fraction per gram of original soil. Data could not be expressed per unit weight of size/density fraction because the amount of contaminating polytungstate could not be determined. The organic carbon or nitrogen content of the two middle density fractions (1.85–2.07 and 2.07–2.22 g cm⁻³) of any size fraction was calculated by subtracting the amount of organic carbon or nitrogen in the density fraction from that in the next lighter density fraction.

Potentially mineralizable nitrogen was also estimated for the size/density fractions and for intact aggregate fractions. The floatation and separation procedure was the same as described in the preceding paragraph except size/density fractions were not dried prior to incubation. The moist fraction plus the glass fiber filter were placed in 30 ml of N-free nutrient solution adjusted to pH 7.0 (Catroux and Schnitzer, 1987) in a wide-mouth 250 ml Erlenmeyer flask and the incubation chambers were inoculated with 1 ml of pre-incubated soil suspension. Flasks were closed with neoprene rubber stoppers and incubated at 26°C for 28 days on an orbital shaker rotating at 56 rpm. We calculated the amount of O₂ available in the head-space of the incubation flasks to be about 2500 µmol. The metabolic demand of the size/density fractions never exceeded 50 µmol of O₂ over the course of a 28 day incubation and we therefore assume the mineralization potential estimates were entirely aerobic. We determined that sodium polytungstate does not interfere with nitrogen mineralization after a short initial lag, although it does appear to inhibit nitrification. Baseline mineral nitrogen values were determined by extracting duplicate moist size/density fractions with 15 ml of 2M KCl. After 28 days, the incubated organic matter fractions were extracted with 2M KCl and mineral nitrogen was assessed.

RESULTS

The small macroaggregate fraction (250–2000 µm) obtained from capillary-wetted soil from three tillage treatments (High Plains Agricultural Research Station, Sidney, NE, USA) contained 52–60% of the total soil organic carbon and 51–68% of the total soil organic nitrogen (Table 1). No-till soil had more organic carbon and nitrogen associated with macroaggregates than

TABLE 1

Effect of tillage on the fraction of total soil C and N in the macroaggregates

Tillage treatment	Total soil N and C (%)	
	N	C
Bare fallow	51	52
Stubble mulch	58	55
No-till	68	60

TABLE 2

Effect of tillage on the fraction of total soil N and C in the enriched labile fraction (ELF)

Tillage treatment	Total soil N and C (%)	
	N	C
Bare fallow	9	20
Stubble mulch	16	18
No-till	25	18

any other treatment. These data suggest that no-till management can improve or maintain the macroaggregate structure of cultivated soil and can increase the ability of the soil to sequester organic carbon and nitrogen compared to conventional management practices.

Eighteen percent of the total soil carbon and 25% of the total soil nitrogen in no-till soil was associated with fine-silt size particles having a density of $2.07\text{--}2.22\text{ g cm}^{-3}$ isolated from inside macroaggregates (enriched labile fraction or ELF) (Table 2). This pattern of organic carbon and nitrogen accumulation in the ELF was also found in bare fallow and stubble mulch soils. The amount of organic nitrogen accumulating in the ELF decreased as the intensity of tillage increased (Table 2), but organic carbon contents remained unchanged. These data suggest the processes controlling the cycling of organic carbon and nitrogen into and/or out of the ELF are not coupled. For example, it is possible that organic carbon turnover is controlled by physical protection mechanisms while organic nitrogen turnover is controlled by chemical protection.

The specific rate of mineralization (μg net mineral N/ μg total N in the fraction) for macroaggregate-derived ELF was not different for the three tillage treatments, but was greater than that for intact macroaggregates (Table 3), suggesting that the ELF was protected from decomposition within the aggregate structure of the soil. However, we do not know how rapidly the ELF

TABLE 3

Specific rates of mineralization for the ELF fraction and for intact aggregates for the three treatments in the macroaggregates

Tillage treatment	Rate coefficients for N mineralization within fractions ($\mu\text{g mineralized N g}^{-1}/\mu\text{g tot N g}^{-1}) \times 10^{-2}$)	
	Intact aggregates	ELF
Bare fallow	2.8	6.4
Stubble mulch	1.2	5.2
No-till	2.3	2.5

was turning over inside intact macroaggregates. The slower mineralization rate assessed for intact macroaggregates may indicate the presence of a less-labile fraction (or fractions), such that when the respective mineralization rates were averaged, the observed net rate was lower.

CONCLUSIONS

Utilizing the methods for separation of soil organic matter fractions described in this study, we have improved our ability to quantitatively estimate soil organic matter fractions, which in turn has increased our understanding of soil organic matter dynamics in cultivated grassland systems. The data suggest that no-till management may ameliorate the detrimental effects of intensive cultivation by improving aggregate stability and increasing organic carbon and nitrogen accumulation in cultivated grassland soils. We believe the adoption of reduced tillage management could be an important step towards the goal of sustainable production, which optimizes long-term profits to the farmer and minimizes damage to the environment.

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Influence of content and nature of organic matter on the structure of some sandy soils from West Africa

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ABSTRACT

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In the Soudano-Sahelian area, the expansion of more intensively cultivated fields has detrimental effects on both the stock of organic matter and the soil structure. Tropical sandy soils from Mali and Burkina Faso (West Africa) are characterized by low amounts of clay (<20%) and organic matter (<2%) which partly explains their weak structure and intense soil surface structural crusts formation under rainfall.

The study of 20 topsoil samples shows the striking relationship which exists between soil aggregate water-stability and organic matter content.

When the structural stability was relatively high, soil structure was more compact with self-similar aggregates of different sizes. When it was low, quartz was often coated and cemented by organo-mineral material in all the sandy soils studied.

This study also reports investigations about the nature of these organic materials in two pairs of soil samples of contrasting structural stability in water, tensile strength and organic matter content. Organic matter in all the selected soils was characterized by its high degree of humification, with a high proportion of humin fraction (75 to 90%) and low fulvic acids/humic acids ratio. A strong organic-clay association and the incorporation of nitrogen in humic materials were confirmed by the resistance of carbon and nitrogen to acid hydrolysis (70 to 80% of C and N were unhydrolysable). Humic acids were very rich in oxygen-containing functional groups, and poorly condensed, with close similarities to fulvic acids from other soil types. These general organic patterns should be attributed mainly to bioclimatic environment and partly to the expansion of continuous cultivation.

Results supported that the structural stability of the sandy top-soils studied could be the result of both the special chemical nature of organic matter with numerous functional acid groups and its strong association with goethite-kaolinite within the clay-size fraction. Low soil organic matter contents were mainly explained by the low amount of clay mineral-organic matter associations and limited

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even more by intensive agricultural practises. The most stable soils were characterized by large contents of humin, uronic acids, osamines and polyphenols, which would be related to soil microbial activity. It was finally suggested that, among these substances, uronic acids produced the strongest aggregative effects.

INTRODUCTION

Organic matter content is considered as a major factor affecting soil structure, especially in tropical soils (Strickling, 1950; Combeau et al., 1961; Combeau and Quantin, 1964). In more recent studies a positive correlation between organic matter content and aggregate stability in various soil types and management has been reported (Tisdall and Oades, 1982; Chaney and Swift, 1984; Bartoli et al., 1988).

In their pioneering work Combeau and Quantin (1964) and more recently Dormaar (1983) suggested that the stability of soil aggregates is more likely related to the arrangement of the organic matter than to the amount of soil organic carbon. Both polysaccharides (Guckert, 1973; Cheshire, 1979; Tisdall and Oades, 1982; McKeague et al., 1986) and humic substances (Combeau and Quantin, 1964; Vicente and Robert, 1981; Chaney and Swift, 1986) are known to play a major role in aggregate stabilization. Nevertheless the contribution of each of them has not yet been clearly established in all soil types (Chaney and Swift, 1984).

In sandy soils of West Africa low organic matter content is generally attributed to (i) their clay contents being lower than 20% (Fauck, 1972; Pieri, 1989), and (ii) overgrazing and intensive farming (Pieri, 1989; Pallo and Thiomballo, 1989). Soil structural stability also decreases with decreasing clay content (Fauck, 1972; Pieri, 1989) and with increasing period under intensive cultivation (Combeau and Quantin, 1963).

The objective of the present study is to assess the role of organic matter on aggregate stability in these tropical soils, more specifically with respect to the content and specific properties of (i) humic substances and (ii) non humic substances, such as polysaccharides and N-containing biomolecules.

MATERIAL AND METHODS

Soils

The study areas are located in the Soudano-Sahelian savanna zone in the Saria's agronomic station (Burkina Faso) and in the Molobala area (Mali) with respectively granodiorite and sandstone as parent rock materials. The climate is tropical with a mean annual temperature of 35°C during the dry season (October–April) and 25°C during the wet season (May–September).

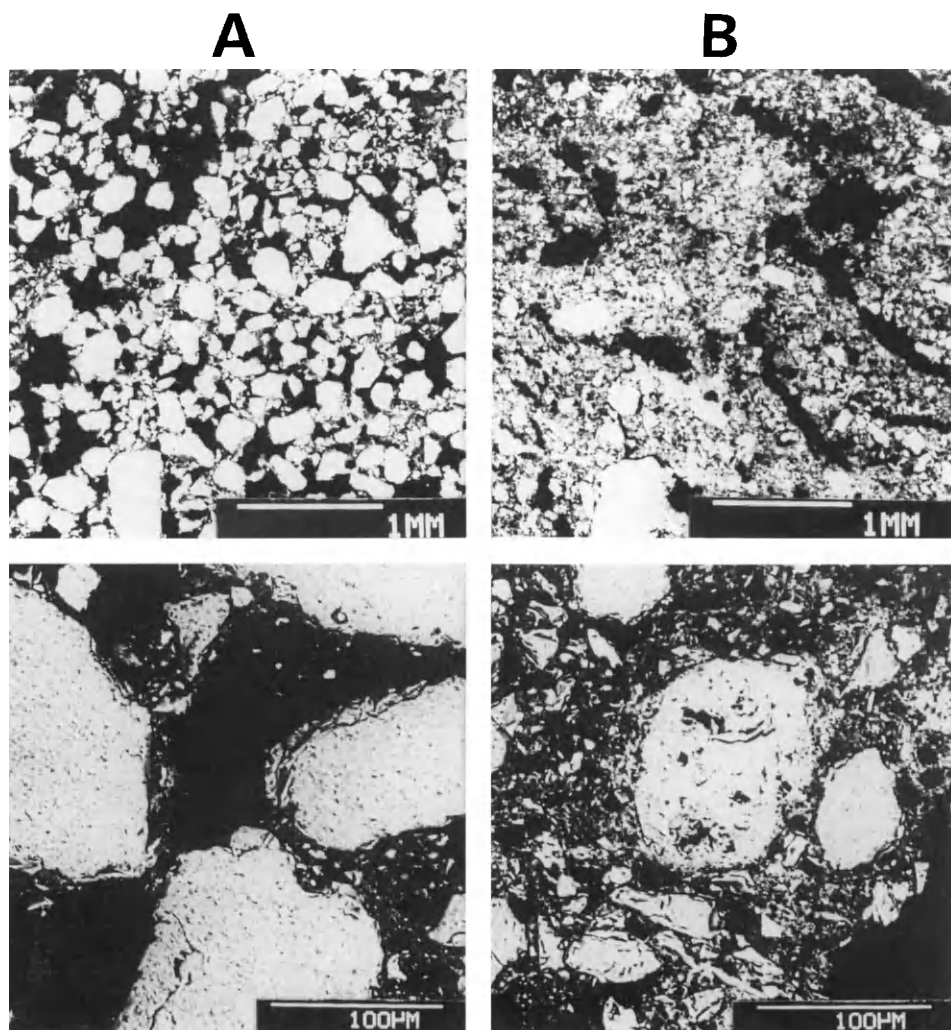


Fig. 1. Electron scanning micrographs of thin sections of top soils from Mali (A) and Burkina Faso (B) observed by the backscattered electron technique.

The mean annual precipitation is 820 mm in Burkina Faso and 1100 mm in Mali.

Twenty soils were sampled near the end of the dry season, mainly in May 1989, from the upper 5–15 cm, under the ridges in the field, of ferruginous tropical soil (Alfisol (USA)–Luvisol (FAO)). For a good sampling standardization the 0–5 cm layer, perturbed by soil ploughing and surface crusting, was excluded. Soils were cultivated with *Sorghum bicolor* and *Pennis-*

TABLE 1

Main characteristics of four selected Ap horizons

Ap 5–20 cm characteristics	Samples			
	Mali		Burkina Faso	
	A	B	C	D
<i>Particle size (%)</i>				
0–2 μm	4.3	7.3	25.3	8.1 a 17.2 b
2–20 μm	5.1	7.0	10.8	18.1 5.8
20–50 μm	10.9	35.1	16.6	11.5
50–200 μm	45.7	37.5	20.4	16.1
200–2000 μm	32.9	11.0	21.8	45.7
pH (H ₂ O)	5.24	4.92	5.56	5.19
S (meq/100 g)	1.44	1.84	7.48	3.33
Organic C %	0.35	0.36	0.97	0.56 a 0.22 b
Organic N %	0.026	0.026	0.068	0.69 0.038
C/N	13.5	13.8	14.3	18.2
Fe _{CBD} (‰)	2.6	5.2	42.4	54
Fe _{Ox} (‰)	0.1	0.1	0.8	0.9
Fe _{CBD-Ox} (‰)	2.5	5.1	41.6	53.1
Al _{CBD} (‰)	0.6	0.8	4.4	5.4
Al _{Ox} (‰)	0.5	0.5	1.1	1.1
Al _{CBD-Ox} (‰)	0.1	0.3	3.3	4.3

a = <0.1 μm .b = 0.1–2 μm .

tum typhoides in Burkina Faso and with *Sorghum bicolor*, *Pennisetum typhoides* and *Gossypium sp.* in Mali.

The samples were air-dried and passed through a 2 mm sieve prior to analysis. These samples were characterized by their low content of 2–20 μm fine silt ($6.8 \pm 3.3\%$), 0–2 μm clay ($13.7 \pm 6.4\%$), crystalline Fe oxyhydroxide (citrate–bicarbonate–dithionite extractable iron (Fe_{CBD}) less oxalate extractable iron (Fe_{Ox}) = $0.52 \pm 0.23\%$), principally as goethite, and organic carbon ($0.39 \pm 0.22\%$) (Fig. 1, Table 1). Consequently, bases were low (3.59 ± 1.76 meq/100 g) and quartz was the dominant mineral in the sand and silt fractions, whereas poorly crystalline kaolinite (determined by hydrazine X-ray test and infrared spectroscopy) was the predominant clay mineral. Two pairs of soils samples of contrasting structural stability in water, designed A to D (Table 1), were selected for this preliminary study. Only one

of them (C), located in a Vertisol area, was an exception in having smectite in the clay fraction.

Physical and chemical analysis

The oxalate extractable iron and aluminium (Fe_{Ox} and Al_{Ox} respectively), which are attributed to poorly-ordered iron and aluminium hydrous oxides, were determined by atomic absorption spectrometry after dissolution with oxalic acid/ammonium oxalate, for 3 h at 20°C and in the dark, as described by Tamm (1922). The citrate–bicarbonate–dithionite extractable iron and aluminium (Fe_{CBD} and Al_{CBD} respectively) were determined after dissolution for 30 min at 80°C, as described by Mehra and Jackson (1960).

The pH values of the soil suspensions were determined in water and in 1.0 M KCl, using a soil:solution ratio of 1:2.5.

The cation exchange capacity (CEC) was measured by atomic adsorption spectrometry after exchange with 0.5 M NH_4Cl .

Soil structure

Soil thin sections were prepared using the method developed in the Centre de Pédologie Biologique, Vandoeuvre, France (Doirisse, 1989), with epoxy resin, and were observed with a light microscope and with a Cambridge scanning electron microscope. Contrast images were obtained using the backscattered electron microscopy technique, introduced for application in soil science by Bisdom and Thiel (1981).

Ultra-thin sections of the 0–0.1 μm and 0.1–2 μm clay fractions were prepared following the procedure described by Villemin and Toutain (1987) and observed by transmission electron microscopy (TEM), using a JEOL 200 CX microscope.

The Thiéry cytochemical reaction (1967) was also used in order to stain polysaccharides selectively on these sections.

Soil tensile strength

The air-dried 0–2 mm samples were put into 3.5 cm diameter and 1.4 cm high moulds and thereafter water equilibrated at pF 2, compressed at 0.5 kg/cm² and air-dried following the procedure described by Hoogmoed (1985). Tensile strength was determined by crushing soil pellets between two parallel plates (Dexter, 1975; Hoogmoed, 1985). Soil tensile strength measurements were carried out in the Soil Tillage laboratory of Wageningen Agricultural University (the Netherlands).

Aggregate water-stability

Aggregate water-stability tests were carried out with 0–2 mm air-dried samples following the method described by Bartoli et al. (1991a). Two grams of sample was added to 200 ml distilled water in a sieving machine with 9 brass 200 μm sieves, designed in the Centre de Pédologie Biologique, Vandoeuvre, France.

Particles not associated with sand or aggregate passed through the sieve. The amount of water-stable > 200 μm aggregates was obtained by subtracting the sand fraction, obtained after Na resin dispersion, from the quantity of soil material retained on the sieve.

Some preliminary disaggregation kinetics were carried out showing that the sieving time of 1 hour (water stable aggregate after 1 hour = WSA 1 h) was an appropriate characteristic of sandy soil structural stability in water.

Amounts of clay and of organic carbon were determined on some WSA 1 h.

Particle size distribution and soil physical fractionation

Ten grams of soil were suspended in 200 ml distilled water and shaken for 2 hours in an end-over-end shaker at 40 rpm. The sand fraction was separated by sieving at 100 μm and dried at 105°C. The 0–50 μm fraction of each sample was added to 200 ml distilled water and 100 ml IR 77 Amberlite (Na^+) resin used as dispersing agent (Rouiller et al., 1972; Bartoli et al., 1991b). From this suspension, the separation of silt (2–50 μm) and clay (0–2 μm) fractions was carried out by sedimentation, pipetting by Robinson's method, centrifugation. Each fraction was finally dried at 105°C.

Since more than 80% of organic material was found in the 0–20 μm fraction (Table 2) the following procedure was carried out in order to concentrate organic material before analyses. A volume of 100 ml IR 77 Amberlite (Na^+) resin suspension was added to 10 g of soil in a polyethylene bag, and shaken with 200 ml distilled water for 2 hours in an end-over-end shaker at 40 rpm. The separation of the fractions was then obtained by wet-sieving.

Finally, after Na-resin methodology, the separation of the clay suspension into < 0.1 μm and 0.1–2 μm size classes was made on samples C and D using a Sharpless ultra centrifuge (Rouiller et al., 1984).

Humic and fulvic acids of the Na-resin soil 0–20 μm fraction

Ten grams of 0–20 μm fraction were suspended in 200 ml of 0.1 M sodium hydroxide, shaken for 1 hour and centrifuged for 10 min at 10.000 g. The extraction was repeated on the centrifugation sediment from 5 to 8 times. The humin fraction dispersed in the extract was flocculated with 1.0 M KCl. In the supernatant the fulvic (FA) and humic (HA) acids fractions were sepa-

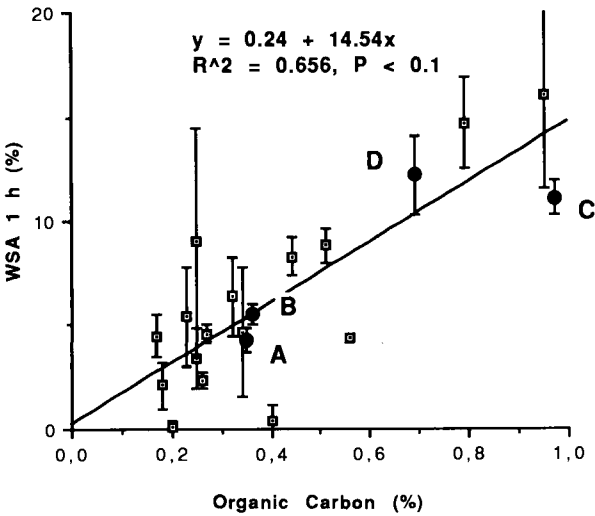


Fig. 2. Relationship between water-stable aggregate (WSA 1h) and soil organic matter content. (□) Topsoil, (●) samples A, B, C and D selected for organic matter analysis.

TABLE 2

C/N ratios and organic carbon contents of the different particle-size fractions of the 4 soils selected

Sample:	Organic carbon: % of fraction				Organic carbon: % of total organic C				C/N			
	A	B	C	D	A	B	C	D	A	B	C	D
<i>Particle size (%)</i>												
2-50 μm	0.55	0.19	0.32	0.31	22.8	25.0	19.6	21.7	14.7	18.0	15.0	15.4
0-2 μm	5.11	4.08	3.27a	2.21a	43.0	85.7	58.0a	32.9a	12.2	13.2	12.6a	12.3a
			2.81b	2.15b			23.5b	10.0b			11.7b	11.3b

a = <0.1 μm.

b = 0.1-2 μm.

A, B=samples from Mali.

C, D=Samples from Burkina Faso.

rated by acidification to pH 1.5 with 2.0 M HCl and centrifugation. The separated fractions were then purified by dialysis, decationization on Dowex WX8 (H⁺) resin, and finally freeze dried following the procedure described by Barriuso (1985).

Organic C, H, N and O were determined with a CHN+O Carlo Erba 1104 autoanalyser for solid samples; organic C content of FA and HA solutions was determined by combustion in a Carmhograph 12 Wösthoff.

Estimation of the molecular weight of FA and HA was carried out by gel

filtration on a single column of G 50 Sephadex gel using a pH 7.5 tris buffer as elutant. UV-visible spectra of dissolved FA and HA were recorded on a UV-visible "Beckman 25" spectrophotometer. E_4/E_6 chromatic ratios were determined, using absorption values at 465 and 625 nm.

Hydrolysable carbon and nitrogen forms of the Na-resin soil 0–20 μm fraction

The 0–20 μm soil fractions were first hydrolyzed with 1.0 N H_2SO_4 (50 mg C/100 ml) for 6 hours, according to Cheshire (1979) and Barriuso (1985), and the soil residues were separated by centrifugation. Neutral sugars were analysed, in the supernatants, by the phenol method (Dubois et al., 1956) and by the anthrone method (Brink et al., 1960) using a glucose solution (100 mg/l) as a standard. Uronic acids were determined by the method of Dormaar and Lynch (1962), using glucuronic acid as a standard, and reducing phenols were determined according to Ribereau Gayon (1962), using protocatechic acid as a standard.

Hydrolysis with 3.0 N HCl (50 mg C/100 ml) was carried out according to Janel et al. (1979). Nitrogen in α -amino-acids and osamines were determined colorimetrically, using a leucine solution as a α -amino-acid standard (Moore and Stein, 1954) and a glucosamine solution as an osamine standard (Sowden, 1959), respectively.

RESULTS AND DISCUSSION

Soil structure

Soil micrographs showed two types of interactions between quartz and organo-mineral plasma. They are herein described, according to Bullock et al. (1985)

(i) A gefuric to chitonic related distribution for the A and B soil samples from Mali. These soils had a low content of both clay plasma and iron oxides (Table 1) which can only act as coatings on the quartz surfaces (Fig. 1A). In these cases soil structure was quite homogeneous and was characterized by an important macroporosity. Surface structural crusts (Casenave and Valentin, 1989) were also a characteristic feature for the unstable A and B soils samples.

(ii) A close and open porphyric related distribution for the C and D soil samples from Burkina Faso. Aggregates were composed of quartz, sandy nodules and compact clay plasma, and were separated from each other by more or less elongated pores (Fig. 1B). Organisation into self-similar aggregates was also characteristic of these samples (Fig. 1B) and was attributed to their relatively large contents of both clays and crystalline iron oxyhydroxides (Table 1).

Aggregate water-stability and tensile strength

Structural stability (WSA 1 h) was mainly correlated with organic carbon content (Fig. 2). The regression equation was as follows:

$$\text{WSA 1 h (\%)} = 0.28 + 14.54 \text{ organic C (\%)} \quad (r=0.66, P<0.1 \text{ for 20 samples})$$

As previously noticed, the two pairs of soil samples (A–B and C–D) were selected on the basis of their contrasting aggregate water-stability and organic matter content. Correlation between the amount of clay and WSA was not so well established ($r=0.15$).

Tensile strength results confirm the contrast between the low stability of the A–B soil pair ($10.2 \times 10^3 \text{ N/m}^2$ and $28 \times 10^3 \text{ N/m}^2$ for the A and B soils respectively) and the higher stability of the C–D soil pair ($197 \times 10^3 \text{ N/m}^2$ and $72.6 \times 10^3 \text{ N/m}^2$ for C and D soils respectively).

Total organic carbon content

The C–D soil pair had about twice as much organic matter as the A–B soil pair (Table 1).

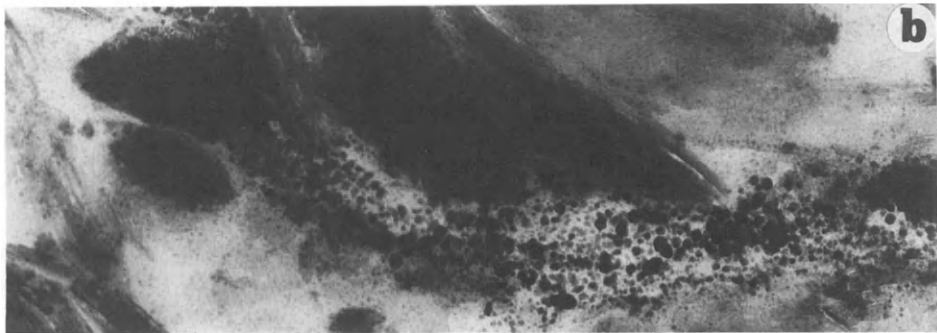
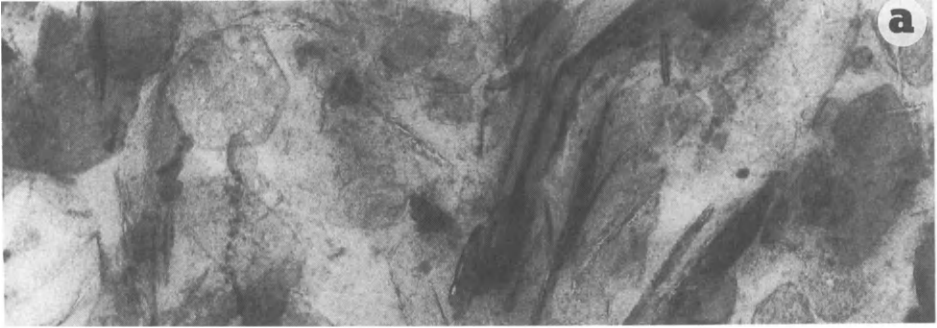
The tropical soils studied herein contained less carbon (0.35 to 1%) than usually reported for the savannas. For instance Sanchez et al. (1982) found that the average soil carbon and nitrogen contents was 1.68% and 0.153% respectively for 61 topsoil samples from various parts of the tropics. In West Africa as well, organic carbon usually ranged from 1 to 2% (Feller et al., 1983; Dabin, 1988; Pallo and Thiomballo, 1989; Pieri, 1989). The small contents of organic C and N measured in the studied soils could be attributed to intensive farming without residue recycling, which has occurred in these areas for 10–15 years (Ayodele, 1986).

Distribution of organic matter in the different particle-size fractions

The content of organic carbon increased with decreasing particle size (Table 2). This confirms previous results on various soils by numerous authors (e.g. Andreux et al., 1980; Bartoli et al., 1988; Elliot et al., 1991).

The carbon content in the size fractions largely depends on their mineralogical composition, mainly the nature of clay minerals and metal oxides. In Australian Alfisols, Jocteur Monrozier et al. (1991) recently attributed the smaller carbon and nitrogen content in the 0–0.1 μm fraction than in the 0.1–2 mm fraction to the larger amount of oxyhydroxide present in the latter. In the present study, TEM showed that the fine 0–0.1 μm clay size fractions were organised in elementary units of organo–mineral crystallites, whereas the coarse 0.1–2 μm fraction contained kaolinites–Fe oxyhydroxides and organic matter associated into microaggregates (Fig. 3, Table 1). Thiéry's cytochemical reaction test for polysaccharide revealed as electron dense organic matter,

A



B



Fig. 3. Ultra-thin sections of clay 0–0.1 μm (a) and 0.1–2 μm (b) fractions from Burkina Faso soil samples observed by TEM and stained by the Thiéry cytochemical agent (1967). A=Soil sample C; B=Soil sample D.

in the 0.1 to 2 μm diameter fraction (Figs. 3Ab and 3Bb), the abundance of polysaccharides. According to Foster et al. (1983), other organic molecules enriched with OH groups could also react with lead.

The C/N ratios also decreased with particle size, from 15 to 18 in the silt size fraction to 12 in the clay size fraction, as is usual in soils. These values were relatively large when compared to the average values of 8–9 reported by Jocteur Monrozier et al. (1991) under tropical conditions. These values are probably affected by the rate of humification: as a matter of fact, recent seasonal analyses carried out on the same soils showed that the C/N ratios of the clay fractions were near 10 at the beginning of the dry season, but progressively increased up to 14–15 at the end of this season (Dutartre, unpubl. data). The presence of low N content plant species could be another explanation but no measurement has been done until now.

Alkaline extraction of organic matter

Extraction, limited to the 0–20 μm size fraction, showed that the main proportion of soil carbon was present in the humin (unextractable) part of the organic matter fraction (Table 3). In soils A, B and D, the percent of humin C and N were $69\% \pm 2$ and $69.5\% \pm 4.7$ of total organic carbon and nitrogen, respectively. There was a significant positive correlation between humin carbon (hC) and humin nitrogen (hN) content ($r=0.94$; $P<0.05$).

The higher values of percent of humin C and N for sample C possibly arose from its large clay content, especially the presence of montmorillonite with high cation exchange capacity and specific surface area. In the tropics, about 45 to 60% of the soil organic C and 45 to 65% of the total nitrogen were reported to be in the humin fraction (N'Guyen and Duchaufour, 1969; Moyano et al., 1991). In the same soils in Burkina Faso, Dabin (1988) found that humin C was near to 60% of total organic carbon. The larger values observed in the present paper were obtained on the less than 20 μm fraction which does not contain the coarser mineral fractions. High proportion of organic matter as unextractable form indicates a high degree of maturity of the organic matter in the finer soil fractions, and a strong binding between fine inorganic components and organic matter.

In soils A, B and D, humic acids (HA) represented $24.1 \pm 1.3\%$ of the total organic C. The lowest value of 5.5% was obtained from soil C. Fulvic acids (FA) in soils A, B and D were less abundant and represented $6.9 \pm 3\%$ of the total organic C and a similar value was obtained for N. Consequently, the $C_{\text{FA}}/C_{\text{HA}}$ and $N_{\text{FA}}/N_{\text{HA}}$ ratios were much lower than 1.0 confirming the high degree of humification of the first three soils. In the case of sample C, the FA/HA ratio was higher, although the FA contents was lower than in other soils. As stated by Jocteur Monrozier and Duchaufour (1986), low values of FA would indicate a high polymerisation degree with possible transformations of

TABLE 3
Percent of 0–20 µm organic C and N in humin, humic and fulvic acid fractions

Sample	Total organic carbon in the 0–20 µm fraction (%)	% of total organic carbon		Total nitrogen in the 0–20 µm fraction (%)	% of total nitrogen			
		hC	HAC		hN	HAN	FAN	FAN/HAN
A	0.35	66.5	24.1	0.026	67.8	24.4	7.8	0.32
B	0.36	69.3	22.9	0.026	65.7	27.5	6.8	0.25
C	0.97	89.7	5.5	0.068	92.8	5.0	2.2	0.44
D	0.69	70.8	25.5	0.038	74.8	19.4	5.8	0.30

hC= Carbon content in humin, HAC= Carbon content in humic acid and FAC= Carbon content in fulvic acid.
hN= Nitrogen content in humin, HAN= Nitrogen content in humic acid and FAN= Nitrogen content in fulvic acid.

TABLE 4

Physico-chemical characteristics of humic acids

Sample	Analysis				Ratio		
	C (%)	H (%)	N (%)	O (%)	C/N	C/H*	C/O*
A	49.98	4.13	4.55	41.34	10.98	1.01	1.61
B	48.09	3.93	3.99	43.99	12.05	1.02	1.45
C	45.60	3.99	4.12	46.29	11.07	0.92	1.32
D	47.71	3.81	3.95	44.54	12.08	1.01	1.43

*Atomic ratio.

FA into HA. But the low amount of FA could also result of lower adsorption of FA on smectites, resulting in their higher mobility out of the soil (Vicente and Robert, 1981).

Humic acids in the four soils had quite similar analytical properties (Table 4). Their oxygen contents were high, and their aromaticity, as revealed by their H/C atomic ratio which was close to 1. The C/N ratios in humic acids from the 0–20 μm fraction were lower than those of the whole soils and size separates. The E_4/E_6 ratios were lower than those published by Volkoff et al. (1988) on tropical savanna soils in Brazil. This seems to indicate the predominance of HA with low condensation rate. Using gel filtration (G 50 Sephadex gel) only a small difference was observed between the four humic acids. Each of them contained a predominant fraction excluded from the gel columns, with an average molecular weight larger than 10.000 daltons and an other fraction with an average molecular weight ranging between 4.000 and 5.000 daltons. Due to this similarity, no relationship could be found between E_4/E_6 values and the proportion of condensed aromatic material (Piccolo, 1988).

Acid hydrolysis of organic matter

Carbon distribution (Table 5)

A comparison was made between the unextracted soil 0–20 μm fraction and the corresponding humin 0–20 μm fractions. The predominance of unhydrolysable carbon (UNC), especially in the humin was noticed in all cases. The highest values were obtained in the B and C soils, and the lowest ones in the A and D soils.

From 40 to 50% of hydrolysable C was identified as sugar molecules. Neutral sugars (NS) predominated, especially in soils A and D and in their corresponding humin fractions. However, when NS were calculated as a percentage of the total sugar fraction, their proportion decreased from 70% in the A

TABLE 5

Distribution of sugars, phenols, α -amino acids and unknown carbons forms in the unextracted 0–20 μm fractions and in the corresponding humin fractions

Sample	Distribution in unextracted soil % Total organic carbon				Distribution in humin % Total organic carbon					
	Total sugars		Phenols	α - amino acids	UHC	UNC	Total sugars			
	Uronic acids	Osamine Neutral sugar					Uronic acids	Osamine	Neutral sugar	α - amino acids
A	4.4	3.7	13.2	4.8	22.5	47.0	3.0	1.9	12.3	2.2
B	1.9	0.9	5.9	2.0	18.6	69.6	3.3	1.7	8.1	2.0
C	4.3	2.5	6.5	5.6	20.4	57.7	5.5	2.5	9.3	2.2
D	9.4	4.8	11.5	9.4	15.0	47.6	9.6	5.5	9.7	2.5

UHC = unknown hydrolysable carbon.
UNC = unknown non hydrolysable carbon.

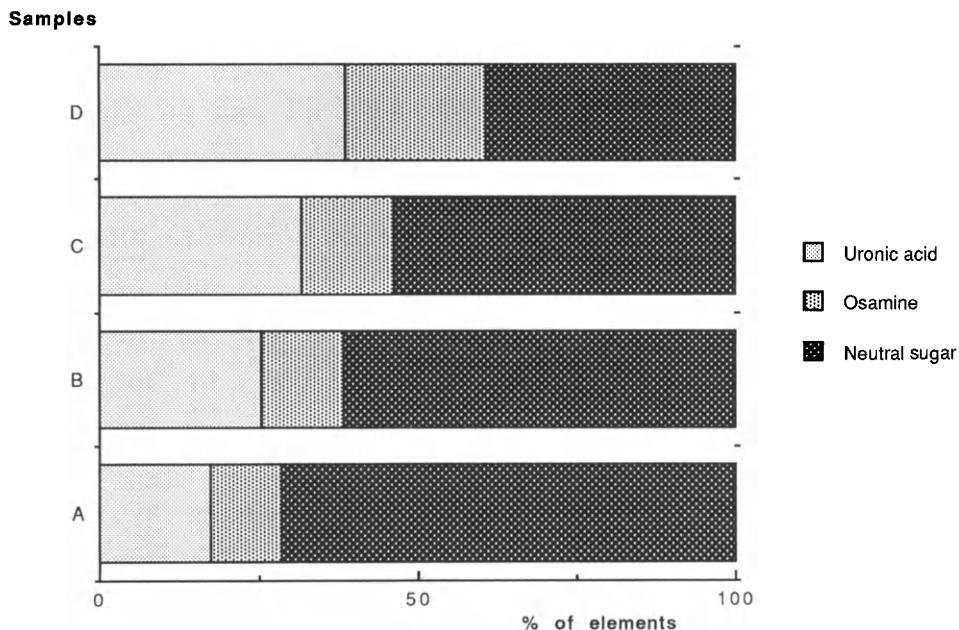


Fig. 4. Relative distribution of sugar forms in the 0–20 μm humin fractions.

soil to about 40% in the D soil. This was even more pronounced in the humin fractions (Fig. 4). The proportion of osamins (OA) and uronic acids (UA), especially the latter ones, increased conversely, and was therefore much higher in aggregated soils from Burkina Faso (C and D soil samples) than in aggregated soils from Mali (A and B soil samples). These results are in agreement with data of Visser (1968) and Gückert (1973), showing an increasing influence of sugar on aggregation with $\text{NS} < \text{OA} < \text{UA}$. The strong aggregative properties of uronic acids (UA) was related to their ability to form salts and complexes with metals and cationic surfaces (Hamblin and Greenland, 1977).

Amino acid carbon was poorly indicative in this respect. Hydrolysable phenolic carbon was shown to increase sharply with aggregate stability, particularly in the humin fractions. The nature of hydrolysable organic materials from soil or from humin confirms the relationship of both acidic polysaccharides and phenolic molecules to soil aggregate stability.

Nitrogen distribution (Table 6)

Only small differences were found between the unextracted 0–20 μm soil and the corresponding humin fractions. In both extracted and unextracted soils incorporation of organic nitrogen in polycondensed molecules was shown by the presence of 30 to 50% of unhydrolyzable N. In tropical soils, this fraction rarely exceeds 10–15% of soil N (Jocteur Monrozier and Andreux, 1981). The NH_4^+ -N proportion ranged from 6 to 13% of soil N and resulted partly

TABLE 6

Distribution of organic nitrogen compounds in the unextracted 0–20 μm fractions and in the corresponding humin fractions

Samples	Distribution in unextracted soil % Total nitrogen				Distribution in humin % Total nitrogen			
	NH ₄ ⁺	Osamine	α - Amino acids	UNN*	NH ₄ ⁺	Osamine	α - amino acids	UNN*
A	6.6	4.5	40.8	48.1	12.7	4.8	38.0	44.5
B	6.3	3.3	30.5	55.4	7.4	4.5	40.5	47.6
C	6.8	5.3	57.5	30.4	8.7	9.5	39.7	42.1
D	8.3	9.5	37.0	45.2	6.3	11.1	39.6	43.0

*Unknown non hydrolysable nitrogen.

from the desorption of exchangeable ammonium partly from the splitting of amino groups from aminosugars and amides during the process of hydrolysis.

Contribution of α -amino-acid-N generally did not exceed 40% of total N. No clear relationship with the clay content could be noted. α -amino-acid-N is in higher proportion in many soil types (Schnitzer, 1991). The proportions of osamine-N were higher in the humin than in the extractable fractions, as already reported by Jocteur Monrozier and Andreux (1981), and ranged between 4.8% of total N in A and B soils, and 9.5–11.1% in C and D soils. Osamin-N represents commonly 6 to 9% of soil-N in tropical soils (Schnitzer, 1991), and a high content of osamine-N was related to soil clay content and aggregation (Gallali, 1972).

The above results confirm that a higher proportion of osamins was related to a higher clay content in soil, and could therefore contribute to the higher aggregate stability of the two soils from Burkina Faso.

CONCLUSION

The amount of organic matter in the fine fraction ($<20 \mu\text{m}$) of soil, in close relation to the amount of clay-size components, largely controls soil aggregate stability. Clay minerals and organic materials are strongly associated into organo-mineral microaggregates (plasma) which cement sands into larger structural units when decomposed organic matter content is high. When organic matter is low or undecomposed, soil constituents are more thinly surrounded by the plasma. Using a description according to Bullock et al. (1985) in the first case, a porphyric related distribution with a good structuration is created, whereas in the other case, only a less stable gefuric enaulic distribution, can appear.

The chemical nature of soil organic matter appears to be involved in the

stability of the structure in the studied soils. Humic substances with large molecular weight and a high proportion of aromatic materials could exert a strong binding effect to mineral components. This aggregative effect was also confirmed in other tropical soils in Africa (Feller et al., 1983; Piccolo et Mbagwu, 1990).

Amongst the biomolecules, uronic acids and osamines play the main role rather than total sugars. In the humin fraction especially, the formation of salts or complexes with metals and surface cations made unextractable the biopolymers which contain such molecules and contribute to maintain aggregate cohesion in these soils. As a whole, the association between humic substances, uronic acids and osamines largely contributed to both soil structure and aggregate water-stability in these tropical sandy soils from West Africa.

In order to demonstrate the influence of these organic components on the soil structural stability in water other experiments are needed. We are currently investigating texture and chemical characteristics of water-stable aggregates (WSA). Preliminary results indicated that 50 to 80% of both clay and organic carbon was found in the WSA, confirming the aggregative role of clay-organomineral associations. Other experiments, in progress, are devoted to the study of soil structural stability in water as a function of water aggregate content, obtained at well defined pF values. Organic matter content associated to clay also induced an increase of the maximum WSA content in the WSA vs H₂O curves.

Finally, it would be suggested to follow simultaneously soil desaggregation and sugar degradation kinetics in water.

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Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere: preliminary examination

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ABSTRACT

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The present paper reports the results of a combined physical and microbial approach to studying the aggregation state of a silt loam soil in the wheat rhizosphere. Non-rhizosphere soil aggregate size distribution was attributed to self-fragmentation of clods. In contrast, aggregate size distribution was uni-modal and centered at 0.2–2 mm for soil adhering to wheat roots. On the other hand, inoculation of wheat with a rhizosphere strain of *Bacillus polymyxa* increased the mass of soil adhering to the roots by 57%. Comparison of aggregate size distributions suggested a more porous structure for the inoculated rhizosphere soil than the uninoculated. An avidin–biotin indirect ELISA procedure, which allows the detection of *B. polymyxa* in its natural environment, indicated that the population remained at a constant level whatever the treatment or the size of aggregates. Aggregate stability in water, which was low, showed no statistical difference between treatments or between aggregate size fractions. The water-stable aggregates studied here represent the elementary rhizosphere aggregates characterized by the association of clays, silts, fine sands and *B. polymyxa* cells.

No effect on the population level of *B. polymyxa* or on the stabilization of aggregates was observed after inoculation of soil and seeds. However, a more porous structure developed within the rhizosphere soil. We are continuing to investigate this aspect of the soil structure process which could be related to a spatial extension of the rhizosphere soil. Inoculation by *B. polymyxa* could play an important role in water retention and nutrient transfer in the rhizosphere through increasing porosity.

INTRODUCTION

Soil structure is important in fertility and plant growth, and may be affected by physical and biological factors. It is well known that organic resi-

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dues applied to soils improve their structures (Allison, 1973). In cropping, the rhizosphere constitutes one of the principal sources of organic material in soils. It contributes to soil organic matter directly through the root material itself and its decomposition, and indirectly through stimulation of microbial activity in the rhizosphere.

Guckert et al. (1975) suggested that plant products such as mucigel could adsorb clay particles and thus may be a mechanism leading to the formation of aggregates. Tisdall and Oades (1979), attributed the efficiency of rye-grass in aggregate stabilization to the fungal hyphae population it supported. Reid and Goss (1981) found that increased aggregate stability of soils associated with root growth of rye-grass and alfalfa was due to polysaccharides produced in the rhizosphere. So far, no attempt has been made to distinguish the effects of the roots from the one due to the associated microbial biomass.

Interest in the use of polysaccharide-producing organisms to improve structure in agricultural soils has increased. Several authors have studied the addition of polysaccharides (Chenu et al., 1987) or exopolysaccharides (EPS-) producing organisms (Lynch, 1981; Metting, 1986; Molohe et al., 1987) to the bulk soil. But in terms of exploiting the native microorganisms in soil or rhizosphere, few attempts have been made to improve EPS production and aggregate stability (Chapman and Lynch, 1985; Thomas et al., 1986a,b; Roberson et al., 1991).

However, soil microbiologists have described bacterial populations which could play a major role in rhizosphere soil aggregation. Using transmission electron microscopy (TEM), Faull and Campbell (1979) have provided excellent pictorial evidence that a rhizosphere colonist of wheat, *Bacillus cereus* var. *mycoides*, is surrounded by an electron-transparent region, presumably polysaccharide, to which clay particles adhered. Among *Bacillus* species isolated from the rhizosphere of *Graminae*, the presence of the nitrogen-fixing bacteria *Bacillus polymyxa* has been also reported (Neal and Larson, 1976; Lindberg and Granhall, 1984; Mavingui et al., 1990). This species has been tested for its growth-promoting effects on *Graminae* (Holl et al., 1988) but no author has reported an effect on soil aggregation in the rhizosphere.

The present paper reports the results of a combined physical and microbial approach to studying the aggregation state of a silt loam soil in the wheat rhizosphere. The aim of the study was to investigate the short-term effect of inoculation with the rhizobacteria *B. polymyxa*, on rhizosphere soil structure.

MATERIALS AND METHODS

Soil

A silt loam soil (Eutric Cambisol, 36.8% sand, 48.1% silt, 11.6% clay), pH 5.6, from eastern France (near Nancy) was used in this study. The soil sam-

ple was collected in November 1989 from the homogeneous Ap horizon (0–30 cm) which was previously under a rape crop. Clay minerals were illite, vermiculite, kaolinite and goethite. Cation exchange capacity by 0.5M ammonium chloride was 7.4 meq per 100 g. Air-dried soil contained 0.7 g kg⁻¹ N and 10.2 g kg⁻¹ organic C. The soil sample (moisture 15% w/w) was stored up to 3 months in plastic containers at room temperature.

Bacteria and bacterial inoculum

Bacillus polymyxa strain CF43 was isolated from rhizosphere soil samples containing roots of spring wheat seedlings (*Triticum aestivum* L., cv. Castan). Plantlets were grown in a silt loam soil (Eutric Cambisol) from a cultivated area near Nemours, France. The size of the *B. polymyxa* population ranged from 1 to 5 × 10⁵ bacteria (g dry weight)⁻¹ of rhizosphere soil. *B. polymyxa* strains were identified by morphological and biochemical tests. In the presence of sucrose, strain CF43, which was the most efficient N₂-fixing *B. polymyxa* isolated, produced two types of exocellular polysaccharides, heteroglycan acid (polymer of glucose–galactose–fructose and uronic acid) and levane, a neutral fructose polymer of low molecular weight (10⁴ to 10⁵ monomeric units). Stock cultures of *B. polymyxa* CF43 were stored in their polysaccharides at –80°C. A pure culture of *B. polymyxa* strain CF43 was grown in a modified Luria Bertani broth at 30 ± 2°C for 16 to 18 h, i.e. until the end of the exponential growth phase. The number of bacteria was adjusted to 10⁴ CFU ml⁻¹ in 0.1M sterile KCl solution. A 50 µl-volume of suspension was plated onto nutrient agar (Biokar) and grown for five days at 30 ± 2°C. Sporulating bacterial colonies were scraped from the agar plates and then washed twice in 0.1M KCl solution. Bacteria were harvested by centrifugation (3600 × g for 30 min) and the final concentration of the inoculum adjusted to 10⁸ bacteria ml⁻¹ in distilled water.

Inoculation and obtention of rhizosphere soil

Soil and wheat seeds were inoculated by a spore suspension of *B. polymyxa* CF43. Polypropylene pots (8 cm diam. and 40 cm length) were filled with 1.7 dm³ of unsieved moist soil equivalent to 2.6 kg dry weight of soil. The top (5 cm) layer of the soil was inoculated with the bacterial spores. The spore suspension was sprayed at the rate of 45 ml per kg of soil. It was calculated that each gram of dry soil received 5 × 10⁶ CF43 spores. Control soil was treated similarly with distilled water. Wheat seeds were inoculated by mixing them with the bacterial suspension. Gum arabic (15%) was used to improve seed bacterization. Control seeds were treated with gum arabic and distilled water only. Bacterized and control seeds were then air-dried for 48 h at room temperature. The seed population of strain CF43 was estimated by serial di-

lution plating before sowing. About 5×10^6 CFU of strain CF43 per seed were detected in inoculated samples and none in uninoculated seeds. Three seeds of wheat, *Triticum aestivum* cv. Fidel were sown in each pot. There were three plots with nine replicate pots for each treatment. The pots were transferred to a glasshouse and were watered per ascensum with distilled water. The plants were harvested after 3 weeks. The intact root-soil mass was removed from the pot and handled gently while carefully entangling the roots. The soil adhering strongly to the roots was referred to as rhizosphere soil. The rhizosphere soil was carefully separated from the roots using a fine silk bristle brush and tweezers. To obtain sufficient soil for analyses it was necessary to bulk the rhizosphere soil samples from the nine pots of each plot. Soil samples were air-dried and then stored at 4°C. The plant material was separated into roots and shoots and the seed was excised. Roots were washed briefly in water before storage at -20°C and shoots were dried at 60°C.

Aggregate size distribution and water-stability

The rhizosphere soil was dry-sieved into seven aggregate-size fractions, <0.1 mm, 0.1 to 0.2 mm, 0.2 to 0.5 mm, 0.5 to 1 mm, 1 to 2 mm, 2 to 4 mm and >4 mm. The dry weight of the soil was estimated for each fraction. Total C and N contents of the soil were determined on triplicate samples of each fraction using a CHN 1106 Carlo Erba Analyser.

Water-stability of the aggregates was studied on 2 g of each of the >0.2 mm air-dried fractions added to 200 ml distilled water in a wet sieving machine designed in our laboratory (Bartoli et al., 1991). Nine sieves (height 70 mm, diameter 60 mm, mesh size 200 µm) were supported by a 600 mm diameter holder which was vibrated at a steady rate by a motor. Each sieve was immersed in distilled water up to 2 cm and oscillated. The horizontal oscillations, applied for 1 h, were sinusoidal with an amplitude of 2 cm at 100 rpm and a frequency of 1.6 Hz. Each sample was treated in triplicate. Each fraction <0.2 mm was eliminated and there was no cross contamination of samples. Water-stable aggregates >200 µm were calculated by subtracting the >200 µm fractions (coarse sand) obtained after a total disaggregation with Na resin (Rouiller et al., 1972) from the fraction remaining on the sieve.

Extraction of Bacillus polymyxa from aggregates and wheat roots

Washed roots from inoculated and control plants were cut into pieces, and samples (0.1 g) were homogenized by a crusher (MM2 – Retsch) in 10 ml of phosphate-buffered saline (PBS): NaCl, 7.2 g l⁻¹; KH₂PO₄, 0.4 g l⁻¹; Na₂HPO₄, 2.8 g l⁻¹ (final pH 7.2). The slurry obtained was used for coating wells in microtiter plates.

Rhizosphere soil samples (1 g dry weight) were suspended in 50 ml of PBS,

pH 7.2. The suspension was dispersed by ultrasonic probe with an outside energy of 50 W as determined by the procedure described by North (1976). Dispersed soil suspensions received 200 μ l flocculant solution (720 mg SrCl_2 , 6 H_2O l^{-1} , resulting concentration: 0.01 mM SrCl_2), shaken vigorously by hand for 2 min and allowed to settle for 15 min. The supernatant was used for coating wells in microtiter plates (100 μ l per well). Three replicates of each sample and a control without antiserum were used.

Detection of Bacillus polymyxa: the ELISA procedure

Immunoassay reagents and equipment

Phosphate-buffered saline (PBS): NaCl, 7.2 g l^{-1} ; KH_2PO_4 , 0.4 g l^{-1} ; Na_2HPO_4 , 2.8 g l^{-1} (final pH 7.2). Tris-buffered saline (TBS): NaCl, 8 g l^{-1} ; Tris base, 0.604 g l^{-1} ; HCl (1M), 3.8 ml l^{-1} (final pH 7.6). Washing buffer: TBS plus 0.05% Tween 20 (v/v). Blocking solution: washing buffer supplemented by 1% (w/v) bovin serum albumin (Sigma). Substrate buffer composition per liter was as follows: diethanolamine (Prolabo), 97 ml; pH was adjusted by HCl 12 N (final pH 9.8). Biotinylated donkey-antirabbit antibodies (RPN 1004) and streptavidin conjugated with alkaline phosphatase (RPN 1234) were purchased from Amersham. Absorbance at 405 nm of hydrolysed substrate (4-nitrophenylphosphate ref. 107905, Boehringer Mannheim) was measured with an automatic microplate reader MR 5000 (Dynatech Laboratories). Polystyrene microplates Nunc Maxisorp F96 (ref. 4-39453) were used.

Antiserum production

Bacteria were grown in a modified Luria Bertani broth (tryptone 10 g l^{-1} , yeast extract 5 g l^{-1} , NaCl 5 g l^{-1}) for 24 h at $30 \pm 2^\circ\text{C}$ in a rotary shaker (200 rpm). Cells were harvested by centrifugation at $10,000 \times g$ for 20 min at 4°C and washed three times in sterile phosphate-buffered saline (PBS), pH 7.2, and their number was adjusted to 10^9 cells ml^{-1} . Whole cells of *B. polymyxa* CF43 were used to elicit antibodies in (New Zealand $\times$ Californian) rabbits by immunization with multiple intradermal injections. Antisera were collected by bleeding via cardiac puncture and stored at -20°C . Before using, the antiserum was tested for its ability to induce agglutination of strain CF43, as described by Carpentier (1965). The antiserum used in this study had an initial titer of 1:2560. All the strains isolated from wheat rhizosphere and belonging to *B. polymyxa* species cross-react with this antiserum. The absorbances obtained with the closest species, *Bacillus macerans*, *Bacillus circulans* and *Bacillus subtilis* were ten-fold weaker than that generated by *B. polymyxa* CF43 and there was no cross-reaction with the Gram-negative species usually isolated from the soil and the wheat rhizosphere.

Avidin-biotin complex incorporation into ELISA (AB-ELISA)

Each well of a microtiter plate was coated with 100 μl soil bacteria suspension in PBS pH 7.2 and allowed to dry at 40°C. The plate was then washed three times with TBS-Tween. The washing buffer was supplemented with 1% bovin serum albumin and added to the wells to block unreacted sites. After 1 h at 37°C, the wells were washed as described above. Wells were then filled with 100 μl rabbit anti-CF43 serum diluted at 1:10,000 in TBS-Tween and incubated for 3 h at 37°C. After all unbound antibodies were washed off, 100 μl biotinylated donkey-antirabbit IgG was applied to each well. The biotinylated IgG was previously diluted to 1:1000 in the blocking solution. Plates were incubated at 30°C overnight. After three washings, each well received 100 μl streptavidin alkaline phosphatase conjugate diluted to 1:1000 in TBS-Tween. The plate was incubated at 37°C for 3 h and washed again twice with TBS-Tween and once with TBS pH 7.6. Then 100 μl of a freshly prepared substrate solution, 1 mg *p*-nitrophenyl phosphate ml^{-1} in diethanolamine buffer, was added, incubated at room temperature for 30 min and the subsequent development of a yellow color indicated a positive reaction. The enzymatic reaction was recorded as the absorbance at 405 nm, measured with a microplate reader MR 5000 (Dynatech Laboratories). In each set of AB-ELISA tests, wells lacking antigens were included as blanks. There was a significant linear correlation between the absorbance values and the number of *B. polymyxa* cells coated in the wells in the range of 3.8 and 5.5 log bacteria ml^{-1} .

Statistical analysis

The data were analysed using a completely randomized design containing two factors (aggregate-size fractions, and inoculation) and three replicates per treatment. Analysis of variance was performed on untransformed data with the exception of particle-size distribution in water-stable aggregates, which was arc-sine transformed. All statistical analysis were performed using STATITCF a software program by the Institut Technique des Céréales et des Fourrages (ITCF, Paris, France).

RESULTS

Effects of wheat roots on soil structure

The distribution of size fractions obtained by dry sieving was different for root-free and rhizosphere soils (Table 1). There were more macroaggregates in root-free soil as compared to rhizosphere soil. Non-rhizosphere soil aggregate size distribution agreed well with a power-law function. It was attributed to a self-similar fragmentation of clods. In contrast, aggregate size distribu-

TABLE 1

Aggregate size distribution for the root-free and rhizosphere soils studied

Aggregate size fractions (mm)	Root-free soil (%)	Rhizosphere soil (%)
<0.2	1.0	16.8
0.2–2	10.2	40.0
2–4	13.3	19.2
4–10	25.5	24.0
> 10	50.0	0

tion was quite uni-modal and centered at 0.2 mm to 2 mm for soil adhering to wheat roots.

Effect of Bacillus polymyxa inoculation on plant growth and rhizosphere soil structure

Inoculation of soil and wheat seeds with the rhizosphere strain CF43 significantly increased the mass of soil adhering to the roots by 57% (Table 2). However, there was no significant effect of inoculation on shoot and root dry weight after 21 days of growth.

The effect of inoculation on amounts of macroaggregates was very clear (Fig. 1). There was much more soil in the 0.2 mm to 4 mm aggregate-size fractions for inoculated soil. This distribution approached a power-law function. The geometric mean diameter (GMD) as defined by Mazurak (1950), was not significantly larger ($p=0.15$) for the inoculated rhizosphere soil than the control (0.955 ± 0.051 mm and 0.892 ± 0.065 mm, respectively). Comparison of aggregate-size distributions suggested a more porous structure for the inoculated rhizosphere soil than for the non-inoculated one.

TABLE 2

Effect of *Bacillus polymyxa* CF43 inoculation on plant root and shoot dry weight, and weight of rhizosphere soil

Treatment	Shoot dry weight ¹ (g/plant)	Root dry weight (g/plant)	Rhizosphere soil dry weight (g/g root)
Control	0.157 a ²	0.146 a	7.5 a
Inoculated	0.156 a	0.147 a	11.8 b

¹Plant harvested at the beginning of tillering.

²Average of three replicates. In a column, means followed by a common letter are not significantly different at the 5% level.

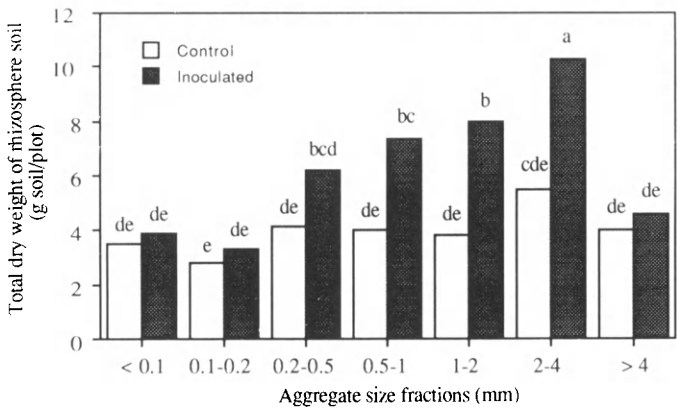


Fig. 1. Effect of inoculation with *Bacillus polymyxa* CF43 on total dry weight of rhizosphere soil occurring in seven aggregate size fractions. The significant differences observed between treatments are recorded by the letters *a*, *b*, *c*, *d* and *e*, as identified by the Newman–Keuls test. Mean values with common letters are not significantly different at the 5% level.

Extraction of Bacillus polymyxa from aggregates and wheat roots

Absorbance at 405 nm obtained with the avidin-biotin ELISA procedure from aggregates and wheat roots largely exceeded the 0.1 absorbance commonly observed as the background level (Table 3). Results confirmed that the native population of *B. polymyxa* was able to colonize rhizosphere soil and wheat roots. The level of *B. polymyxa* population per gram dry weight of soil was similar regardless of inoculation treatment. The *B. polymyxa* population estimated by ELISA procedure was the same in the different aggregate size fractions whatever the treatment, whether inoculated or not (Table 3). Comparison of absorbances obtained from the 2% (w/v) rhizosphere soil and 1% (w/v) roots suspensions suggested no substantial difference between *B. polymyxa* colonization levels of rhizoplane or soil adhering to the root.

TABLE 3
Evaluation of the effect of *Bacillus polymyxa* CF43 inoculation on root and rhizosphere soil density of *Bacillus polymyxa*, measured by the ELISA method (absorbance at 405 nm per gram)

Treatment	Root	Aggregate-size fractions (mm)						
		<0.1	0.1–0.2	0.2–0.5	0.5–1	1–2	2–4	> 4
Control	1.24a	1.20a	1.20a	1.28a	0.88a	1.12a	1.25a	1.29a
Inoculated	1.25a	1.12a	1.19a	1.18a	1.17a	1.27a	1.29a	1.26a

Average of three replications. In a column, means followed by a common letter are not significantly different at the 5% level.

Chemical characteristics of aggregates

The distribution of total organic C concentrations measured in the various aggregate-size fractions were in good agreement with a normal-law function with a maximum for the 0.5 to 1 mm macroaggregate-size fraction (Table 4). Amount of total C decreased significantly ($p=0.001$) after inoculation regardless of aggregate size. Similar differences between aggregate-size fractions were also observed for N concentrations with the exception of greater amounts of N in the finest aggregate-size fraction than in the coarser one. In contrast, inoculation had no effect on the amount of total N in the rhizosphere soil. The C/N ratio was therefore larger in macroaggregates than in microaggregates and was smaller in inoculated rhizosphere soil than in non-inoculated. There was relatively more C lost by inoculation than N. The improved structuring, which could be related to a spatial extension of the rhizosphere soil, reduced C concentration in aggregates by dilution of root exudates. The flow of nitrogen from the soil to the rhizosphere, did not seem to have change after inoculation.

Aggregate water-stability and particle-size distribution

Water stability of dry sieved aggregates was small (Table 5) and showed no statistical difference between inoculation treatments ($p=0.15$) and between aggregate-size fractions ($p=0.18$). The largest value was measured in the

TABLE 4

Nutrient concentrations and C/N ratio in seven aggregate size fractions of rhizosphere soils

Aggregate size fraction (mm)	Treatment	Total organic C (g kg ⁻¹)	Total N (g g ⁻¹)	C/N
<0.1	Control	10.4 c	1.1 bc	9.42 d
	Inoculated	9.4 c	1.0 bc	9.15 d
0.1–0.2	Control	11.2 bc	1.1 b	10.18 cd
	Inoculated	10.0 bc	1.1 b	9.41 cd
0.2–0.5	Control	11.2 bc	1.0 bc	10.80 bc
	Inoculated	10.4 bc	1.1 bc	9.77 bc
0.5–1	Control	13.3 a	1.2 a	11.07 b
	Inoculated	12.4 a	1.2 a	10.09 b
1–2	Control	11.8 b	1.0 bc	12.18 a
	Inoculated	11.0 b	1.0 bc	10.61 a
2–4	Control	11.2 bc	0.9 c	12.07 a
	Inoculated	10.4 bc	0.9 c	11.20 a
>4	Control	10.1 c	0.8 d	11.93 a
	Inoculated	9.0 c	0.8 d	11.31 a

Average of three replications. In a column, means followed by a common letter are not significantly different at the 5% level.

TABLE 5

Characteristics of water-stable aggregates from rhizosphere soil of control and inoculated plants

Treatment	Aggregate fractions (mm)	Water-stable (% aggregate fraction)	Particle-size distribution		
			Sand (%)	Silt (%)	Clay (%)
Control	0.2–0.5	12.0 a	34.7 a	49.3 a	16.0 a
	0.5–1	7.8 a	29.2 a	46.1 a	24.7 a
	1–2	8.5 a	26.2 a	53.8 a	20.0 a
	2–4	7.7 a	31.4 a	47.7 a	20.9 a
	> 4	8.8 a	28.7 a	51.0 a	20.2 a
Inoculated	0.2–0.5	17.1 a	35.1 a	47.6 a	17.3 a
	0.5–1	12.7 a	24.9 a	56.0 a	19.2 a
	1–2	10.0 a	26.0 a	50.5 a	23.6 a
	2–4	7.9 a	27.6 a	49.0 a	23.4 a
	> 4	9.3 a	34.4 a	43.3 a	22.3 a

Average of three replications. In a column, means followed by a common letter are not significantly different at the 5% level.

smallest aggregate size fraction (0.2–0.5 mm) but was not significantly larger than the other water-stability fraction. Particle-size distribution was obtained after a total disaggregation with Na resin from the water-stable aggregates. Results indicated that the proportion of particles contained in each size fraction did not change (Table 5). The proportion of clay particles was higher than in bulk soil, silt fraction was quite similar and sand particles in lower proportion than in bulk soil. The 0.2–0.5 mm fraction showed the lowest amount of clay.

DISCUSSION AND CONCLUSION

In the rhizosphere, optimal conditions for aggregate formation and aggregate stabilization are combined, since root growth in soil is well known for promoting aggregate stabilization (Allison, 1968; Reid and Goss, 1981).

With regards to soil structure formation, two distinct aggregate size distributions were observed in rhizosphere and non-rhizosphere soils. Our results suggested that the action of the rhizosphere was through fragmentation of the soil mass into smaller units (Table 1). This observed effect of the rhizosphere was similar to those reported by Tri (1968). Two distinct mechanisms of soil structure formation were observed by Tri (1968) in the rhizosphere of forage grasses, depending on the initial physical condition of the soils: (i) granulation occurring either through fragmentation of the soil mass into smaller units, or (ii) through aggregation of smaller units into larger elements. Soil structuration also depends in addition on soil texture, soil moisture content, root

age and the plant species involved (Gilmour et al., 1948; Martin, 1971; Reid and Goss, 1981; Sparling and Cheshire, 1985). The energy with which root samples are shaken to dislodge loosely adhering soil should also be taken into consideration.

The observed effects of different plant species indicated differences in the activities of roots and associated microorganisms. Mycorrhizal infection may play an important role in the formation of soil structure and aggregate stability (Tisdall and Oades, 1979; Miller and Jastrow, 1990). Mycorrhizal infection was not measured in our experiment, but it is unlikely to have been of significance because infection normally takes several weeks to develop (Sanders et al., 1977).

The results of our experiment showed that it was possible to increase the mass of soil adhering to the roots after inoculation with the *B. polymyxa* strain CF43. The change in aggregate abundance was not correlated with either root mass or soil population density of *B. polymyxa* as detected by the ELISA procedure. The change in rhizosphere soil structure suggested that microbial activity (strain CF43 vs. endogeneous *B. polymyxa*), and not the total *B. polymyxa* population size, would contribute to aggregate formation (Harris et al., 1964). The relationship between biomass and the stabilizing effect on soil structuration depends on the microbial species (Lynch, 1984).

The effect of inoculation on soil mass distribution among the aggregate-size fractions was mostly restricted to macroaggregates rather than microaggregates and suggested a more porous structure for the inoculated rhizosphere soil than for the non-inoculated one. Wu et al. (1990) reported that measurements of water retention and pore size distribution for a soil are affected by changes in aggregate-size distribution. Inoculation induced neither increase in population density of *Bacillus polymyxa* nor stabilization of aggregates, but a more porous structure within the rhizosphere soil. The relationship between laboratory examinations and soil structuration in the field is uncertain. However, the inoculation, especially under field conditions, could play a significant role in water retention and nutrient transfer in the rhizosphere.

The absence of a significant effect of inoculation on water-aggregate stability in this short-term study suggested that the binding energy was weak or that the effect was transient, as described by Tisdall and Oades (1982). The role of polysaccharides in the rhizosphere is complex, and Sparling and Cheshire (1985) have reported that the contribution of the recently synthesized carbohydrate in the rhizosphere to the water stability of aggregates was less than that from the older, possibly transformed, carbohydrate in the bulk soil. Particle size distribution suggested that the water-stable aggregates studied here represent the basic rhizosphere aggregates whose structural sub-units could be characterized by association of the clays, silts, fine sands and bacteria (*B. polymyxa*).

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Soil aggregates as microcosms of bacteria–protozoa biota

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ABSTRACT

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Forty-eight air-dried soil aggregates were separately incubated under alternately submerged and dry conditions at 20°C without adding exogenous nutrients. In the course of submersions, the population dynamics of bacteria and protozoa were followed in each aggregate.

It was shown that every aggregate accommodated at least three major groups of bacteria as categorized on the basis of colony forming curve (CFC) analysis. The growth of these bacterial groups occurred in sequence during submersion.

Three to fourteen protozoan groups from each aggregate were detected as active forms. Distribution patterns of these groups among the 48 aggregates differed. The number of groups which were active simultaneously in each aggregate were small and the composition of groups changed gradually in the course of submersion. Frequency of appearance of each group during submersion was different and most groups appeared sporadically. These observations suggest that, in spite of the microbial diversity among aggregates, only a few groups are active concurrently.

INTRODUCTION

A variety of bacteria and protozoa inhabit pores in soil aggregates (Hattori and Hattori, 1976; Kilbertus, 1980; Bamforth, 1985; Coleman, 1985; Vargas and Hattori, 1986; Darbyshire et al., 1989) and, therefore, each aggregate composes a microcosm of bacteria–protozoa biota. With regard to the diversity of microcosms, it has been observed that the numbers of total and gram-negative bacteria vary from aggregate to aggregate (Hattori, 1973), so that, even at the same place in a field, the composition of microorganisms is not uniform among aggregates. It has also been observed that the five major groups

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of protozoa (flagellates, amoebae, small ciliates, *Colpoda* spp. and large ciliates other than *Colpoda* spp.) were heterogeneously distributed themselves among 330 aggregates (Vargas and Hattori, 1990).

The aim of this work is to study: (1) the diversity of the bacteria–protozoa biota among aggregates, (2) population changes in each aggregate.

To study the dynamics of bacteria and protozoa in a single soil aggregate, we incubated each aggregate separately under alternately submerged and dry conditions without adding nutrients. The main features of this experimental system are as follows. (1) As the microbial migration among aggregates is excluded, we can get information on the populations in each aggregate. (2) The only exogenous substance is water agar as the bed material for protozoa. Hence, all nutrients originate from the aggregate. (3) A factor inducing bacterial growth and protozoan excystment is the submersion after drying. Though the submerged state is rather unusual in natural soils, a cyclic change in submersion and drying in this experiment may reflect, to some degree, the changeable moisture content in field soil which is a key factor inducing changes in the microbial population (Foissner, 1987; Kuikman et al., 1991).

MATERIALS AND METHODS

Sandy clay loam containing 2.8% of organic matter was taken from Kumanodou farm near Sendai. The soil was air-dried, sieved to obtain aggregates between 1 and 2 mm in diameter and kept in the laboratory for one year at room temperature. Forty-eight aggregates from this sample were separately put on 1% non-nutrient agar in small wells (16 mm in diameter) on a polystyrene micro-assay plate. Each aggregate was submerged in 1 ml of a sterilized mineral solution and incubated at 20°C. This was the first submersion. The solution was composed of 213 mg of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 54 mg of KH_2PO_4 , 7 mg of KCl, 6 mg of CaCl_2 , 8 mg of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 120 mg of NaCl and 1 l of distilled water. Six times during 29 days of incubation, 1 and 10 μl of liquid portions outside the aggregate were sampled for the study of bacteria and protozoa, respectively. Although these samplings do not give information on the populations inside the aggregates, the population dynamics of bacteria and protozoa may take place mainly in the outer region of the aggregate. We assumed that these dynamics could be estimated by inspecting the surrounding solution.

Aggregates were air-dried at 20°C and, after 20 days of being completely dried, were submerged in 1 ml of sterilized water. Five times during 8 days of this second submersion, 1 and 10 μl of liquid portions were taken again from each well for the study of bacteria and protozoa. We have repeated this cycle of air-drying, water submersion and sampling for nearly one year.

During the first submersion, slaking caused the initial structure of aggregates to disintegrate into micro-aggregates (ca. 0.05–0.25 mm). This micro-

aggregate structure remained as such for a long time after the second submersion.

Using the above samples, the dynamics of bacteria and protozoa in each aggregate were studied. As to the bacteria, 1 μ l of each sample was diluted and inoculated on DNB (100 fold of dilution of nutrient broth) agar plates. Population changes in each aggregate were analyzed by the colony forming curve (CFC) on DNB (Hattori, 1988). It is difficult to say whether or not there is a certain group of protozoa unless the group appears in an active form. For identification of active forms of protozoan groups, 10 μ l of every sample was carefully examined by microscopy at 600 fold magnification. The results of the 1st, 2nd, 3rd and 6th submersions will be described.

The number of samples taken from the surrounding solution of each aggregate were one in the case of the 1st, 2nd and 3rd submersions and two in the case of the 6th submersion. The variation among samples was studied by comparing each of 15 samples from the same well during the 5th submersion.

RESULTS AND DISCUSSIONS

Variation in microbial composition among samples

To examine how large the variation among the samples was, 15 samples were taken from each of the wells of three separate aggregates (15×3) two times (at 24 and 72 hr) during the 5th submersion. The microbial compositions in these samples were then compared. The composition of bacteria was nearly the same among 15 samples from the same aggregate. As to protozoa, some groups could be detected in 70–90% of 15 samples, while other groups were detected less frequently. This suggests that the cell density of the latter groups was low or that these groups excysted less frequently even though they existed in high density. For detection of these groups, careful and repeated inspection is essential.

Composition of bacterial and protozoan groups in a single soil aggregate

According to the development time for bacterial colonies in the CFC of each sample, three major bacterial groups were distinguished: (a) fast growers, (b) intermediate growers and (c) slow growers (Fig. 1). Every aggregate accommodated at least three groups.

From 21 times of microscopic observation of the 48 aggregates during 1st, 2nd, 3rd and 6th submersions, 50 groups of protozoa (23 groups of flagellates, 17 groups of amoebae and 10 groups of ciliates) were detected according to cell morphology. Many of these groups appeared as active forms only once or a few times during submersions, thereby making their identification very difficult. We consequently labelled each group as F1, A1 or C1 etc. The

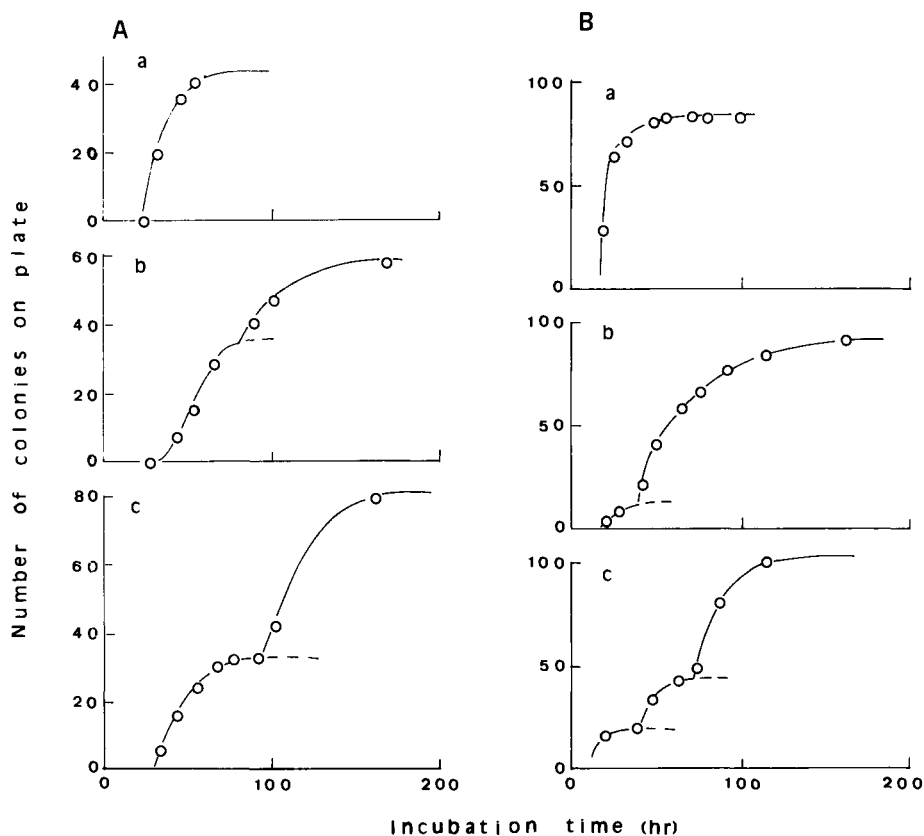


Fig. 1. Change in the composition of bacteria during submersion. Colony forming curves of samples from two separate aggregates (A and B) at the beginning of the 1st submersion are shown. Samples were taken at (a) 28 hr, (b) 4 days and (c) 5 days after submersion.

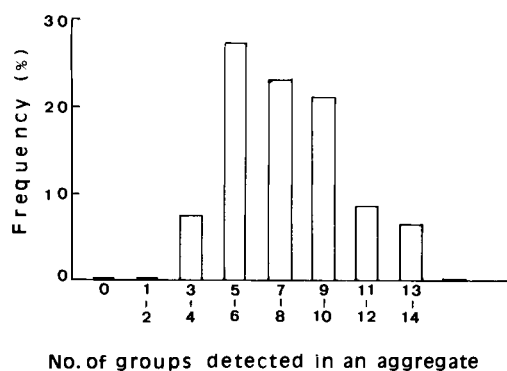


Fig. 2. Diversity of protozoan groups inhabiting separate soil aggregates. Frequency of the number of groups detected as active forms separately in 48 aggregates in the course of 1st, 2nd, 3rd and 6th submersions.

TABLE 1

Protozoan groups which were classified into family or genus level

Flagellates	Amoebae	Ciliates
F1: Chromulinidae	A1: <i>Mayorella</i> spp.	C1: <i>Colpoda</i> sp.
F5: <i>Cyclidiopsis</i> sp.	A2: <i>Vannela</i> sp.	C2: <i>Colpoda</i> sp.
F6: <i>Anisonema</i> sp.	A3: Vahlkampfiidae	C3: <i>Plagiopyla</i> sp.
F7: <i>Phacus</i> sp.	A6: <i>Thecamoeba</i> sp.	C4: <i>Loxophyllum</i> sp.
F10: <i>Cercomonas</i> sp.	A8: Amoebidae	C5: <i>Blepharisma</i> sp.
F13: <i>Petalomonas</i> sp.	A9: <i>Vampyrellidium</i> sp.	C6: <i>Paramecium</i> sp.
F23: <i>Stephanoporos</i> sp.	A10: <i>Diffugia</i> sp.	C10: <i>Colpoda</i> sp.
	A12: <i>Acanthamoeba</i> sp.	
	A16: Hartmannellidae	

Group A4 includes a mixture of unidentified very small amoebae.

groups which could be classified into family or genus level are listed in Table 1.

Every aggregate accommodated protozoa and the number of protozoan groups detected in a single soil aggregate in the course of the 1st, 2nd, 3rd and 6th submersion was 3 at a minimum and 14 at a maximum (Fig. 2). If submersion and microscopic observation are further repeated, both numbers are expected to increase slightly by the addition of newly excysted groups.

The distribution pattern of each protozoan group among the 48 aggregates differed greatly (Fig. 3).

Population changes in a single soil aggregate during submersion

Each submersion after a drying period induced bacterial growth and protozoan excystment in every aggregate.

Bacterial colonies consecutively appeared as indicated in Fig. 1 as an example. In some cases, the total number of colonies increased by the sequential appearance of fast, intermediate and slow growers (Fig. 1A), whereas in other cases, fast growers were gradually replaced by slower growers without increase of the total numbers of colonies (Fig. 1B). The sequential change in bacterial groups was observed in every aggregate, though the detailed composition of each group might not be the same. Microscopic observation of samples showed a gradual change in the morphology of bacteria: (1) huge and long cells (ca. $1 \times 10 \mu\text{m}$), (2) rods, (3) short rods and cocci, (4) spore forming rods with occasional appearance of spindle-shaped or curved rods. The morphological change seemed to correspond to the sequential change in the CFC.

The composition of active protozoan groups in aggregates changed gradually in the course of submersions (Fig. 4). Until four days after the 1st sub-

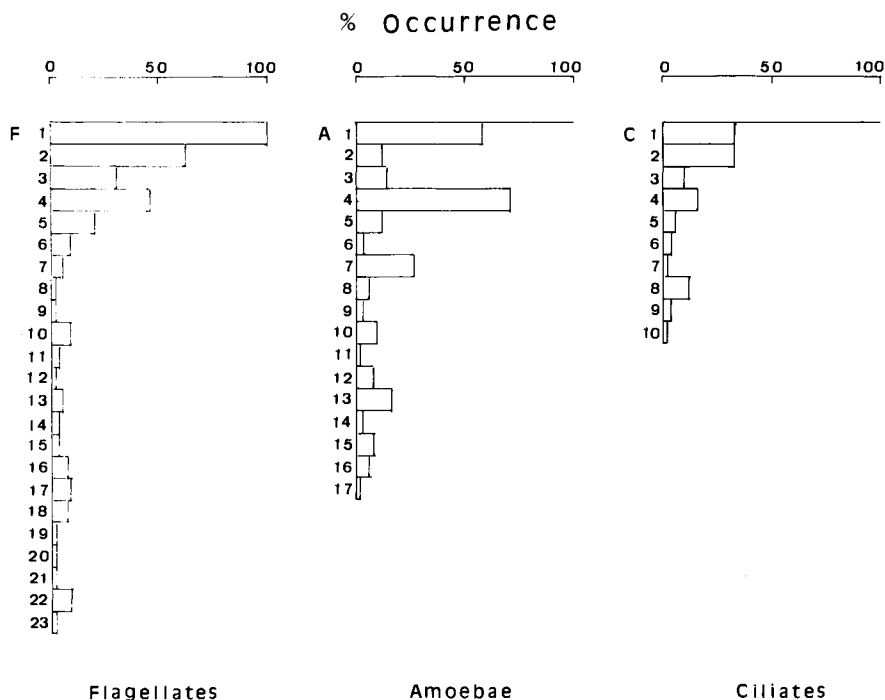


Fig. 3. Distribution pattern of protozoan groups among aggregates. Percentage of occurrence of the respective protozoan group as an active form among 48 aggregates is shown.

mersion, active forms did not appear in 75% of the 48 aggregates but the diversity of active groups increased in the course of the submersion. Similar changes were observed during the 2nd, 3rd and 6th submersions but with shorter intervals of time. It is noticeable in Fig. 4 that only a few groups of active forms appear concurrently, although many more groups may exist in the aggregate. This fact suggests that most protozoan groups inhabiting the same aggregate are encysted. The frequency of excystment may depend on species and environmental conditions. In this experiment, the index of the frequency of excystment (FE) can be formulated as follows:

$$FE = \frac{1}{N} \sum_{i=1}^N (A/S)$$

where N is the number of aggregates, S is the number of times of submersion and A is the number of times the corresponding protozoan group appears as an active form within S times of submersion. The indices for every detected

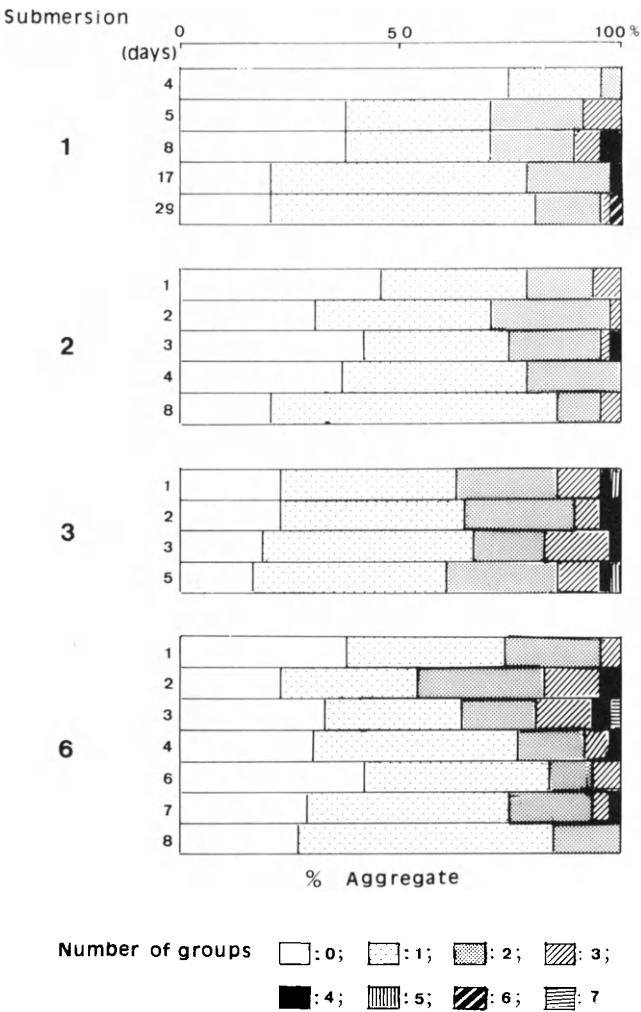


Fig. 4. Change in protozoan fauna during 1st, 2nd, 3rd and 6th submersions. Active forms were not detected in the sample at 28 hr during 1st submersion (data are not shown).

group are listed in Table 2. As shown in the table, the FE value of F1 is exceptionally high, EF values of F2, F3, F4, F10, F22, A1, A4, C1, C2, C3, C4 and C8 are intermediate and FE values of other groups are less than 0.1. The diversity of FE values, along with the diversity of aggregates (Figs. 2 and 3), characterize the population changes of protozoa in the respective aggregates (Fig. 4).

During microscopic observation, the concurrent appearance of active flagellates and rod-shaped bacteria was remarkable. After rod-shaped cells dis-

TABLE 2

Frequency of excystment of protozoan groups in 48 aggregates during 4 submersions

F E	Flagellates	Amoebae	Ciliates
$0.9 \leq FE < 1.0$	F1		
$0.3 \leq FE < 0.4$			C1 C2
$0.2 \leq FE < 0.3$	F2 F10 F22	A 4	
$0.1 \leq FE < 0.2$	F3 F4	A1	C3 C4 C8
$0.05 \leq FE < 0.1$	F5	A7	C5
$0.01 \leq FE < 0.05$	F6 F7 F11 F13 F14 F15 F17 F18 F16	A2 A3 A5 A6 A8 A9 A10 A12 A13 A14 A15 A16	C6 C7 C9 C10
$FE < 0.01$	F8 F9 F12 F19 F20 F21 F23	A11 A17	

appeared, short rods or other types of cells formed aggregates which seemed to be rarely attacked by predators. Deflagellated, inactive flagellates or various kinds of protozoan cysts were often observed within bacterial aggregates and these inactive forms seemed to be re-activated during the next submersion. Large amoebae or ciliates which attack flagellates as well as bacteria appeared only at high densities of flagellates. Such prey-predator relationships may affect the dynamics of bacteria and protozoa in each aggregate.

This work shows that aggregates have divergent compositions of microbial groups. It follows that microbial processes in soil are carried out by a diverse array of microbial assemblages in each aggregate.

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Aggregate stability and soil microbial processes in a soil with different cultivation

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ABSTRACT

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A field trial was started in 1988 to examine the effects of temporary grassland and conventional tillage on aggregate stability and soil microbial processes. Different soil enzymes (actual and potential nitrification, protease, urease, xylanase, alkaline phosphatase) were selected for their importance in recycling N and P for plant nutrition, dehydrogenase and substrate induced respiration (SIR) as characteristics of microbial biomass. Analysis of seasonal patterns showed a significant ($P < 0.05$) increase of SIR and dehydrogenase activity in temporary grassland one year after sowing. Further development of the grassland improved protease activity, xylanase activity and aggregate stability. The following succession can be concluded from the observed results: First, temporary grassland increases microbial biomass, second, microorganisms produce enzymes, which mineralise organic compounds and at least aggregate stability increases due to the influence of microbial biomass, their activities and their by-products. After ploughing grassland in November 1990, both aggregate stability and soil microbial processes decreased rapidly. These results indicate that continuous vegetation and the activities of soil organisms are preconditions to maintain high aggregate stability.

INTRODUCTION

Many studies demonstrate the important relationship of soil organic matter, plant roots and soil microorganisms with aggregate formation of soils (Oades, 1984; Lynch, 1984). Positive correlations of aggregate stability with soil microbial processes led to the general hypothesis, that plant roots and heterotrophic organisms may be important to maintain high aggregate stability in soils. The biotic influence on soil structure can be measured by characterization of soil organisms, their activities and their by-products (Jastrow

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and Miller, 1991). Fungal hyphal length, microbial biomass and root length have been correlated with estimates of water stable aggregate size distribution (Tisdall and Oades, 1980; Gupta and Germida, 1988). Microbial and root exudate polysaccharides were discussed as transient binding agents, humified organic matter and complexes of organic colloids with polyvalent metal cations and clays as persistent binding agents (Tisdall and Oades, 1982; Oades, 1984). The importance of soil microbial processes for aggregate formation in agricultural soils has also been demonstrated by Lynch and Bragg (1985), Gupta and Germida (1988) and Anderson (1991).

Many studies have examined the long-term (10 yr or more) effects of management practices on soil structure and soil microorganisms (Alef et al., 1988; Insam et al., 1991; Roberson et al., 1991), but less information is available on the short-term effects (Haynes and Swift, 1990; Roberson et al., 1991). An understanding of the short-term effects is important to know both which changes to expect in soil and how quickly these changes will appear.

In this study, soil microbial processes and aggregate stability were analysed monthly over the course of two years in temporary grassland and conventional tillage agro-ecosystems. Actual and potential nitrification and various soil enzymes (protease, urease, xylanase, alkaline phosphatase) were selected for their importance in recycling N and P for plant nutrition. Dehydrogenase and substrate induced respiration (SIR) were selected as characteristics of microbial biomass. Analysis of seasonal patterns was necessary to understand the development and extent of soil microbial processes and aggregate stability under different cultivation.

MATERIALS AND METHODS

Experimental field

In 1988 an experimental field with a block design was laid out in Wieselburg (Austria), designed to study the effect of agricultural extensification on soil structure. The soil is classified as a Eutric Cambisol, soil texture loamy silt (24% clay, 68% silt, 8% sand), pH 7.1, carbonate content 6.1%, total C 1.6%. The pH value was determined by glass electrode (soil:0.01M CaCl_2 at 1:5), soil organic matter by the method of Walkley and Black (1934) and carbonate content by Scheibler's method (Thun and Herrmann, 1949).

Meteorological data during the course of the study were recorded by the Institute for Land and Water Management Research, Petzenkirchen, Austria (Fig. 1). The mean annual temperature is 8.6°C, the mean annual precipitation is 708 mm. The area has a Cfb climate according to the Köppen classification. Aggregate stability and soil microbial processes were monitored monthly for two years in two of the treatments, namely: I. No-tillage (temporary grassland). II. Conventional tillage (rotation of maize in 1988, winter

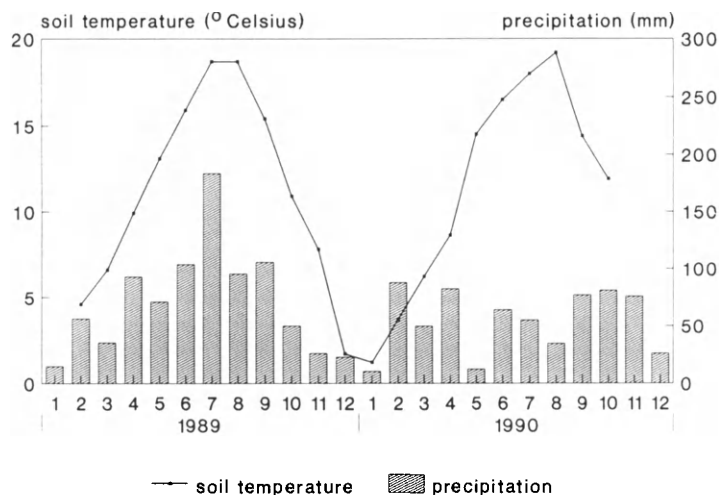


Fig. 1. Meteorological data during the course of the study. The soil temperature was measured at a depth of 20 cm.

wheat in 1989 and spring barley in 1990). The temporary grassland was sown with a mixture of 10% white clover, 17% yellow clover, 33% rough cocksfoot and 40% ryegrass in May 1988. Temporary grassland had not received any fertilization, was mulched three times a year and was ploughed in early November 1990.

The soils from six replicate plots of each treatment (0–20 cm) were collected monthly in a field-moist condition and stored in plastic bags at -20°C . (Heavy overnight frosts and snow prevented soil sampling in January 1990). In addition, samples were taken from an adjacent field with long-term grassland. The permanent grassland was used to represent the baseline level of aggregate development for grassland. After the storage period samples were allowed to thaw at 4°C for about three days. Soil samples were sieved ($<2\text{ mm}$), stored in plastic bags at 4°C and soil microbial analysis was started within two weeks.

The following methods were used for soil microbial investigations:

(1) Substrate induced respiration. An amendment rate of $4\text{ mg glucose g}^{-1}$ soil was found to be adequate for substrate saturation and maximum initial respiration response (Anderson and Domsch, 1978). This rate was therefore used for all substrate additions. The CO_2 evolution was measured according to Jäggi (1976).

(2) Nitrogen mineralisation under anaerobic conditions (Keeney, 1982). The method involves incubation of a 5 g soil sample under waterlogged conditions in an enclosed test tube with little head space. The sample is incubated at 40°C for 7 days, and the amount of $\text{NH}_4\text{-N}$ formed is determined by a colorimetric procedure (Kandeler and Gerber, 1988).

(3) Actual and potential nitrification. Potential nitrification was quantified by measuring nitrite production from soils amended with ammonium and chlorate (chlorate inhibition method; Berg and Rosswall, 1985). Actual nitrification was assayed in the same way, but addition of substrate ($\text{NH}_4^+ - \text{N}$) was omitted.

(4) Activities of soil enzymes: protease (Ladd and Butler, 1972), urease (Kandeler and Gerber, 1988), xylanase (Schinner and von Mersi, 1990), alkaline phosphatase (Hoffmann, 1968). The soil microbial methods and their modifications are described by Schinner et al. (1991).

The following methods were used for physical soil investigations: (1) Aggregate stability. Air-dried samples of the soils were gently sieved to separate 1–2 mm macro-aggregates. Stability of these aggregates was assayed by wet-sieving with the single sieve method (Kemper and Koch, 1966). (2) Water content (ÖNORM L 1062, 1988). (3) Particle-size distribution (Thun and Herrmann, 1949).

Statistical procedure

Data of aggregate stability, microbial biomass and microbial processes in soil are arithmetic means of data from 6 plots. Analysis of variance was performed to determine the variance attributed to the effects of cultivation and season on microbial processes. For regression analysis each single microbiological variable was plotted against aggregate stability. All calculations were made by using the SPSS package.

RESULTS

Microbial biomass followed a similar pattern in the two treatments, the values being lowest during the winter season (cold and moderately dry, Figs. 1 and 2). Time-of-sampling differences were significant for microbial biomass at $P=0.05$. Microbial biomass increased with the age of the temporary grassland. In general, soil under temporary grassland contained significantly ($P<0.05$) higher amounts of microbial biomass than the soil under conventional tillage (Fig. 2). The effect of cultivation on soil microbial biomass was greater in 1989 than in 1990. However, after ploughing the temporary grassland in November 1990, microbial biomass decreased rapidly. Similar results were obtained by estimation of dehydrogenase activity as a measure of active microbial biomass (Fig. 3). The low values of dehydrogenase activity in August 1990 may have been caused by the relatively dry summer. Water content of no-tillage (temporary grassland) and conventional tillage treatments was significantly ($P<0.05$) lower during the summer time 1990 than during spring time 1990 (data not shown).

Figures 4 and 5 show variations in protease and xylanase activity over the

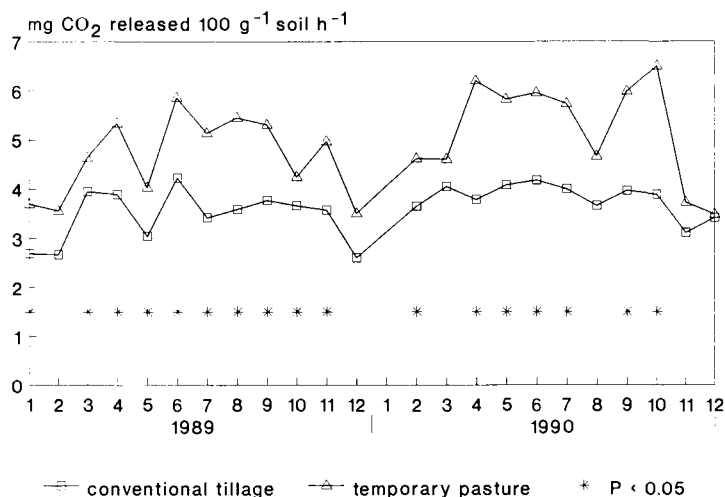


Fig. 2. Seasonal variation of soil microbial biomass (SIR) in conventional tillage and temporary grassland. Asterisks indicate the differences of the two cultivations significant at the $P < 0.05$ level.

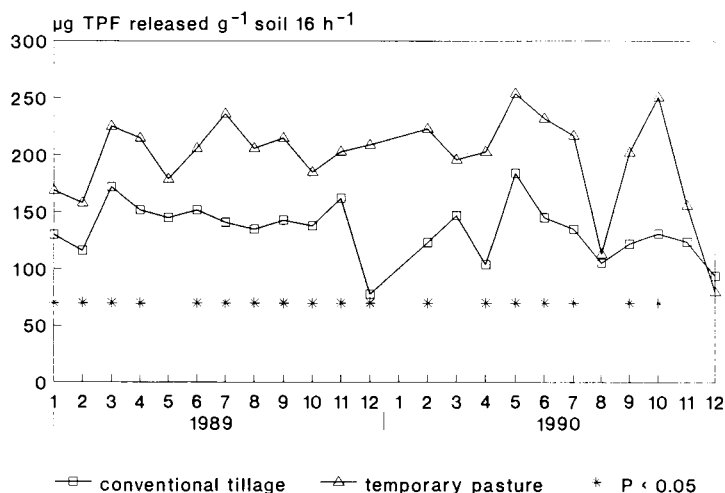


Fig. 3. Seasonal variation of dehydrogenase activity in conventional tillage and temporary grassland. Asterisks indicate the differences of the two cultivations significant at the $P < 0.05$ level.

course of two years. The trends of significance can be identified from the graphs: Xylanase and protease activity showed a number of short-lived peaks during both years. In temporary grassland, enzyme activities increased during the course of study. Differences between the two treatments were significant ($P < 0.01$) during spring and summer of 1990. Ploughing the temporary

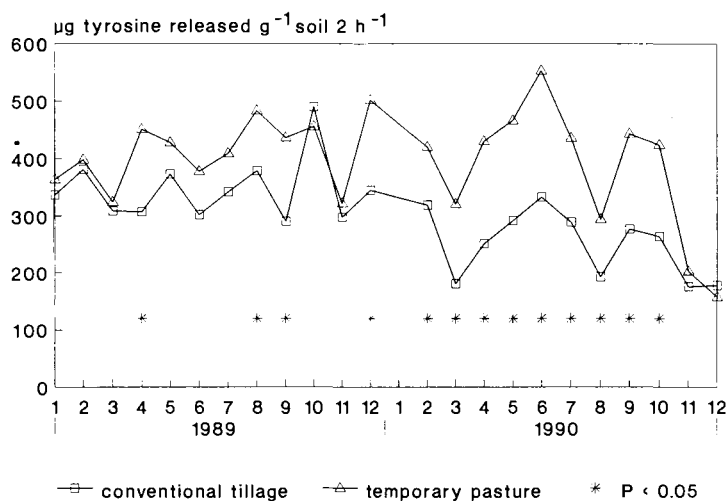


Fig. 4. Seasonal variation of protease activity in conventional tillage and temporary grassland. Asterisks indicate the differences of the two cultivations significant at the $P < 0.05$ level.

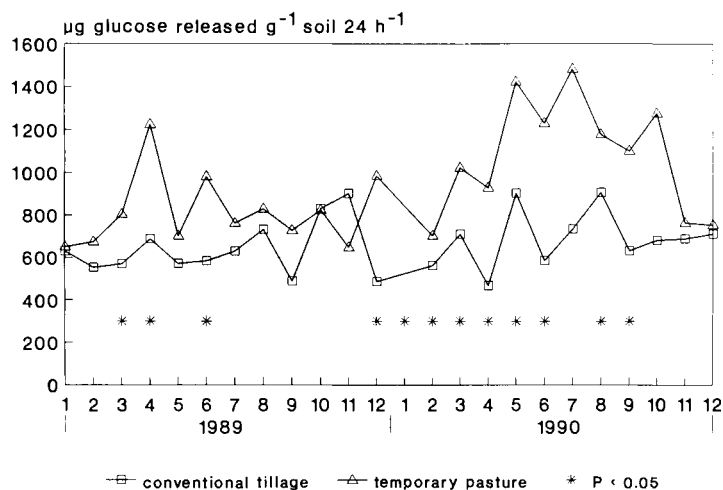


Fig. 5. Seasonal variation of xylanase activity in conventional tillage and temporary grassland. Asterisks indicate the differences of the two cultivations significant at the $P < 0.05$ level.

grassland in November 1990, was associated with a rapid decrease in enzyme activity.

Aggregate stability measured by a wet sieving machine showed a similar pattern (Fig. 6). Differences between the no-tillage (temporary grassland) and conventional tillage treatments were significant ($P < 0.05$) in spring and autumn of 1990. The comparison of this trial with an adjacent permanent grassland indicated, that temporary grassland did not reach the high level of

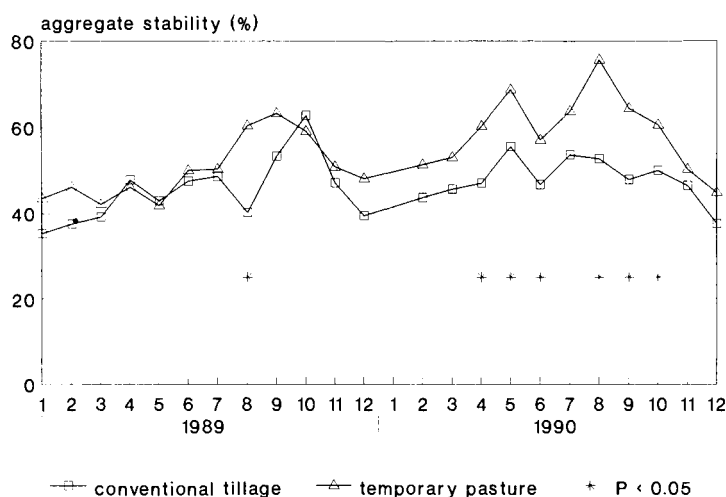


Fig. 6. Seasonal variation of aggregate stability in conventional tillage and temporary grassland. Asterisks indicate the differences of the two cultivations significant at the $P < 0.05$ level.

TABLE 1

Effect of cultivation on aggregate stability, microbial biomass and different microbial processes in a Eutric Cambisol

Soil properties	Conventional tillage ($n = 138$)	Temporary grassland ($n = 138$)	Permanent grassland ($n = 21$)	LSD (0.05)
Microbial biomass ($\text{mg CO}_2 \cdot 100 \text{ g}^{-1} \text{ soil} \cdot \text{h}^{-1}$)	3.6	4.9	6.3	0.7
Dehydrogenase ($\mu\text{g TPF} \cdot \text{g}^{-1} \text{ soil} \cdot 16 \text{ h}^{-1}$)	134	197	322	36
Xylanase ($\mu\text{g glucose} \cdot \text{g}^{-1} \text{ soil} \cdot 24 \text{ h}^{-1}$)	681	947	1018	192
Phosphatase ($\mu\text{g phenol} \cdot \text{g}^{-1} \text{ soil} \cdot 3 \text{ h}^{-1}$)	527	659	1201	129
Protease ($\mu\text{g tyrosine} \cdot \text{g}^{-1} \text{ soil} \cdot 2 \text{ h}^{-1}$)	304	396	624	87
Urease ($\mu\text{g N} \cdot \text{g}^{-1} \text{ soil} \cdot 2 \text{ h}^{-1}$)	36.5	47.4	61.7	7.4
Actual nitrification ($\text{ng N} \cdot \text{g}^{-1} \text{ soil} \cdot 24 \text{ h}^{-1}$)	161	229	308	72
Potential nitrification ($\text{ng N} \cdot \text{g}^{-1} \text{ soil} \cdot 5 \text{ h}^{-1}$)	500	456	477	63
N-mineralisation ($\mu\text{g N} \cdot \text{g}^{-1} \text{ soil} \cdot \text{day}^{-1}$)	45.3	59.8	67.8	9.3
Aggregate stability (%)	46.6	54.8	82.4	24.4

Results are means of 23 collections of soils (cf. Fig. 2 for sampling times), each composed of six replicate samples. For permanent grassland, only 21 collections of samples with triplicate determinations were used.

microbial biomass, enzyme activities and aggregate stability of permanent grassland (Table 1). The potential nitrification was not influenced by cultivation in the Eutric Cambisol. Coefficients of correlation between aggregate stability and various soil microbial processes were significant at $P < 0.001$ (Table 2).

TABLE 2

Coefficients of correlation (r) between aggregate stability, microbial biomass and various soil microbial processes in the samples studied

Microbial processes	Aggregate stability
Biomass	0.58***
Dehydrogenase activity	0.64***
N-mineralisation	0.58***
Protease activity	0.52***
Urease activity	0.61***
Potential nitrification	-0.30 n.s.
Actual nitrification	0.54***
Alkaline phosphatase activity	0.65***
Xylanase activity	0.42***

Asterisks (***) indicate correlations significant at the $P < 0.001$ level.

n.s. = not significant.

DISCUSSION AND CONCLUSION

The general trend in microbial biomass was the same for both conventional tillage and no-tillage (temporary grassland) treatments in the course of two years. Seasonal temperature- and moisture-related effects may be proposed to explain the observed trends. Fluctuation of temperature and moisture have been shown to influence the amount of microbial biomass (McGill et al., 1986; Insam et al., 1989). Furthermore, agricultural practices such as tillage, cropping sequence and residue incorporation influence microbial biomass (Juma and McGill, 1986; Anderson and Domsch, 1986). Our results suggest a fast increase of biomass in temporary grassland as a consequence of changing cultivation from conventional tillage to no-tillage (grassland). Dehydrogenase activity reacts as fast as microbial biomass in soils. The increase of microbial biomass and dehydrogenase activity can be related to the root systems clover-ryegrass compared to maize-wheat-barley. Soil microbial biomass increases with root growth and rooting density (Drury et al., 1991). In addition, differences in rhizosphere microbial populations are known to exist, since plants vary in the amounts and types of organic materials released by their roots (Lynch and Bragg, 1985). Differences in the effect of clover-ryegrass and maize-wheat-barley residues on microbial activities may be related to the narrow C/N ratio of the former. A narrow cover crop C/N ratio is known to favor microbial activities (Rothrock and Hargrove, 1988; Drury et al., 1991).

Soil enzymes are playing an important role in nutrient transformation via biochemical mineralisation (McGill and Cole, 1981; Hunt et al., 1983). The observed increase of enzyme activity in the temporary grassland was probably due to increasing microbial biomass, a major source of enzymes in soil.

However, the relatively slow increase of protease and xylanase activity during 1989 indicates, that the rate of biochemical mineralisation depends on the availability of substrates. Higher amounts of high molecular weight compounds such as carbohydrates and proteins seem to be enriched in a three-year old grassland. In addition, no tillage of the temporary grassland enhances abundant plant residues in the surface layer of soil which are not incorporated by ploughing (Doran, 1987; Saffigna et al., 1989; Dalal et al., 1991).

Microbial biomass is involved in the metabolism of transient binding organic materials such as microbial and plant-derived polysaccharides (Gupta and Germida, 1988). Microbial biomass shows promise as an index of the stability of soils under short-term mixed rotations where soil organic matter remains relatively constant, yet aggregate stability rises during the grassland phase and declines during the arable phase. The following succession can be concluded from the observed results: First, temporary grassland increases microbial biomass by the enhancement of easily decomposable C and N compounds. Second microorganisms produce enzymes which mineralise high molecular weight compounds and at least aggregate stability increases due to the influence of microbial biomass, their activities and their by-products.

Ploughing the grassland reduced aggregate stability and decreased microbial biomass, dehydrogenase, xylanase and protease activity in the 0–20 cm layer. Tillage may bury microorganisms and soil enzymes in a deeper soil layer. In addition, by ploughing the grassland aggregate stability may be reduced by disruption of aggregates, death of microorganisms and/or decomposition of protected organic matter. Nevertheless, the temporary increase of aggregate stability in grassland indicates that permanent vegetation and soil microorganisms are preconditions to maintain high aggregate stability.

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Carbon distribution in different compartments of forest soils

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ABSTRACT

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Three mineral A horizons from forest soils associated with the characteristic forest humus types mull, moder and mor were investigated. The suitability of several physical or chemical fractionation methods for the characterization of major humification processes was determined. The distribution and structures of organic carbon were analyzed after fractionation according to density (plant residues, organo-mineral complexes), aggregate-size (floatables, macro-, microaggregates, clay fraction), and the classical humus fractionation (fulvic acid, humic acid, humin fraction). The fractions obtained were characterized by elementary analysis, wet-chemical methods (CuO oxidation) and CPMAS ¹³C NMR spectroscopy. The classical humus fractionation method seems to produce fractions which are suitable for the investigation of humification processes of lignin in forest soils. The results from both physical and chemical fractionation methods suggest that the presence of soil macrofauna, especially earthworms, is of considerable importance in the formation of organo-mineral complexes and soil aggregates. The structural chemical characterization of individual fractions provides further information about humification processes in soils.

INTRODUCTION

In undisturbed soils, like most forest soils, the equilibrium between litter input and decomposition and humification processes leads to the accumulation of organic matter in distinct forest floor and mineral soil horizons. According to their morphological characteristics the humus types mull, moder and mor are distinguished (SSSA, 1987). The mull humus type is often found on calcareous soils (Rendolls) with a high soil faunal activity and high rate

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of litter decomposition. Consequently it is characterized by a litter layer (L/Oi) directly overlying the mineral A horizon (Arbeitskreis für Bodensystematik, 1985; SSSA, 1987). In contrast, a thick forest floor layer develops on sites with moderate or low faunal activity and slow litter mineralization rates (Schaefer and Schauermann, 1990). These moder or mor humus types are composed of a transitional horizon (Of/Oe), followed by an amorphous organic horizon (Oh/Oa) overlying the mineral soil. Generally, the mull humus type is associated with low C/N ratios of 10–15 in the A horizon, whereas the moder and mor humus types have C/N ratios of 20–29 and > 30 in the Oh horizon, respectively (Duchaufour, 1982). The humification processes in these forest floor horizons (Kögel-Knabner et al., 1990) are different from the processes operating in the mineral soil, where the presence of different mineral soil components such as clay minerals and hydrous oxides strongly influences the retention of organic matter (Martin and Haider, 1986; Oades, 1988). The objective of the present study is to delineate humification processes operating in the mineral A horizons of temperate forest soils.

The conceptual approach includes the fractionation and analysis of forest soil organic matter in different fractions in comparison with the bulk soil. Ideally, these fractions represent compartments of soil organic matter at different stages of decomposition/humification. The samples are fractionated according to chemical and physical properties of the soil. The fractionation procedures chosen are density fractionation, aggregate size fractionation, and the conventional humus fractionation in fulvic acid, humic acid, and humin fractions. The fractions obtained with these fractionation techniques are characterized by elemental analysis, CPMAS ^{13}C NMR spectroscopy and chemical degradation (hydrolysis, CuO oxidation). Thus the organic carbon distribution and structural characterization in chemically and physically defined fractions is obtained. The suitability of the different fractionation techniques to provide fractions which represent distinct compartments of organic matter in forest soils is investigated.

MATERIALS AND METHODS

Samples

Samples were obtained from the forest floor and A horizons of three soils associated with different forest humus types — mull, moder and mor — in the surroundings of Bayreuth, northeastern Bavaria, Germany. Samples were collected on a volumetric basis from about five profiles and pooled. Samples from the organic layers (L, Of, Oh) and the mineral horizons (Ah, Aeh) were freed of living roots as far as possible and air dried. Mineral soil samples were passed through a 2 mm sieve. For chemical analyses of the bulk samples an aliquot of the mineral samples was ground in a ball mill, forest floor samples

TABLE 1

Selected characteristics of the investigated forest soils

Humus type	mull	moder	mor
Main vegetation	European ash <i>Fraxinus excelsior</i>	European beech <i>Fagus sylvatica</i>	Norway spruce <i>Picea abies</i>
Soil type ^a	Cumulic Hapludoll	Typic Dystrochrept	Lithic Udorthent
Sequence ^b	L (4)	L (4)	L (3)
of horizons		Of (6)	Of (3)
(thickness in cm)		Oh (1)	Oh (8)
	Ah (20)	Ah (4)	Aeh (4)
<i>Characteristics of the A horizon</i>			
Texture	loamy clay	sandy loam	loamy sand
CaCO ₃ (%)	19	0	0
pH (H ₂ O)	7.7	4.3	4.2
Eff. CEC (cmol(+) kg ⁻¹)	24.0	8.9	5.7
C _{org} (g kg ⁻¹)	60	70	30
C/N	13	18	21

^aSoil Survey Staff (1975).^bHorizon designations are according to Arbeitskreis für Bodensystematik (1985). They correspond to the Oi (L), Oe (Of), Oa (Oh), A (Ah), and E (Aeh) horizons of the SSSA (1987).

were ground to pass a 0.2 mm sieve. Table 1 gives a description and some chemical characteristics of the forest soils investigated.

Humus fractionation (fulvic acid, humic acid, humin fractions)

Samples from mineral soil horizons of mull, moder and mor were subjected to the conventional fractionation procedure to isolate the operationally defined fulvic acid, humic acid and humin fractions. Ground samples were extracted twice with 0.5M NaOH under N₂ at room temperature at a ratio of 20:1 (NaOH:organic matter). The residue (humin fraction and associated mineral matter) was washed with distilled water and freeze dried. The humic acid fraction was precipitated twice from alkaline solution with 3M HCl, the precipitates were washed with dilute acid and freeze dried. The supernatant solutions (fulvic acid fractions) were combined, dialysed (3500 molecular weight cut off) and freeze dried. The organic C not recovered from the fractionation procedure was 25 ± 12.3%.

Density fractionation (plant residues, organo-mineral complexes)

The A horizons obtained from the forest soils with three different humus types were fractionated by density according to a procedure outlined by

Greenland and Ford (1964). The samples were suspended in bromtrichloromethane/ethanol mixtures with densities of 1.6, 2.0 and 2.4 g cm⁻³ and separated by centrifugation. Each separation step was repeated two to three times. The individual fractions were washed with ethanol to remove bromtrichloromethane and dried at 65°C. The lightest fraction (< 1.6 g cm⁻³) contains fresh or slightly decomposed plant residues, whereas the fractions > 1.6 g cm⁻³, but < 2.4 g cm⁻³ are composed of organo-mineral complexes (Beudert et al., 1989). The morphological composition of the fractions obtained from density fractionation was checked by light microscopy. Organic carbon losses during the fractionation procedure did not exceed 12% of the total organic carbon in a sample.

Aggregate size fractionation (macroaggregates, microaggregates, clay fraction)

The moder A horizon was fractionated into macroaggregates (2000–200 µm), microaggregates (200–20 µm, 20–2 µm) and the clay fraction (< 2 µm) by a combination of wet sieving and sedimentation under gravity. After pre-wetting the bulk soil material for 5 min, separation of macro- and microaggregates (200 µm cutoff) was performed by use of a wet-sieving machine (Retsch, Haan, FRG) vibrating at the lowest possible intensity for 2.5 min. The continuous flow of distilled water through the sprinkling nozzle was set at 1.2 l min⁻¹, corresponding to a total of 3 L of water for a sample of 100 g. The macroaggregate fraction was rinsed off the 200 µm sieve and freed of floating organic material. After removal of floatables also from the suspension that passed the 200 µm screen, the microaggregate and clay fractions were separated by sedimentation in Atterberg cylinders. Dissolved organic carbon (DOC) was separated from the clay fraction by filtration through cellulose nitrate microfilters (0.01 µm). Then all the fractions obtained were freeze dried, weighed and subjected to total carbon and total nitrogen analyses. The loss of organic carbon during the whole procedure amounted to 15%.

Chemical analyses

Replicate analyses were conducted for all chemical data. Total organic C (C_{org}) was determined by dry combustion with a Carmhomat 8 ADG (Wösthoff, Bochum, FRG) for the bulk samples and with a Model 1500 elemental analyzer (Carlo Erba, Hofheim, FRG) for humic, aggregate size and density fractions. The coefficient of variation for both methods is 1%. The pH of the bulk samples was determined in a 1:2.5 (for mineral soil horizons) or 1:10 (for organic horizons) suspension in distilled water. Total nitrogen (N_t) was determined by the Kjeldahl procedure.

Alkaline cupric oxide oxidation was carried out according to the method of

Hedges and Ertel (1982), modified as described by Kögel and Bochter (1985) and Kögel-Knabner et al. (1991). The generated lignin-derived phenols (vanillin, vanillic acid; syringaldehyde, syringic acid; p-coumaric acid, ferulic acid) were separated and quantified by high-performance liquid chromatography (HPLC), using a reversed-phase HPLC technique. The sum of vanillyl (V), syringyl (S) and cinnamyl (C) units (V+S+C), used as an indicator of lignin structures in the humic acid fractions, is given normalized to the aromatic C content of the humic acid fraction as determined by ^{13}C NMR spectroscopy. The mass ratio of acid/aldehyde for the vanillyl units (ac/al)_v can be used to determine the degree of oxidative decomposition of lignin within a sample (Ertel and Hedges, 1984, 1985). The coefficient of variation was 7.6% for the determination of an individual phenol and 5.6% for the calculated acid/aldehyde ratios (Kögel et al., 1988).

CPMAS ^{13}C NMR spectroscopy

Bulk samples and the humic acid fractions were analyzed by CPMAS (cross polarization magic-angle spinning) ^{13}C NMR spectroscopy. ^{13}C NMR spectra were recorded on a Chemagnetics 100S/200L instrument (Chemagnetics Inc., Fort Collins, CO) operating at 25.2 MHz for C. Full details of the methods used are given elsewhere (Hatcher, 1987; Wilson, 1987). Before NMR spectroscopy, the bulk samples and the humic acid fractions from the mineral soil samples were treated with 5M HF for 16 h at room temperature and subsequently washed with 0.1M HCl and freeze dried in order to reduce the content of ash and paramagnetic material. Preston et al. (1989) showed that such a treatment does not effect major structural changes in the composition of humic fractions. The spectra were integrated to determine the percentage of C in the chemical-shift range 0–50 ppm (alkyl C), 50–110 ppm (O-alkyl C), 110–160 ppm (aromatic, olefinic C) and 160–220 ppm (carboxyl/carbonyl/amide C). From the amount of aromatic carbon in the bulk samples and in the humic acid fraction in combination with the yields of C in the humic acid fraction, the amount of aromatic carbon extractable in the humic acid fraction is calculated.

RESULTS AND DISCUSSION

Carbon distribution

Table 2 gives the carbon distribution among the classical humic fractions (fulvic acid, humic acid and humin), as well as the carbon distribution among density (plant residues, organo-mineral complexes) and aggregate size fractions (macro-, microaggregates, clay fraction). Results of the humus fractionation show that the amount of carbon in the fulvic acid fraction is rather low,

TABLE 2

Carbon distribution (expressed as a percentage of the total carbon recovered) in humic, density, and aggregate fractions in mull, moder and mor A horizons

	Mull Ah	Moder Ah	Mor Aeh
<i>Humus fractions</i>			
Fulvic acid	17	12	11
Humic acid	26	50	62
Humin	57	38	27
<i>Density fractions</i>			
Plant residues	13	56	79
Org.-min. complexes	87	44	21
<i>Aggregate size fractions</i>			
Floatables	—	3	—
2000–200 μm	—	23	—
200–20 μm	—	45	—
20–2 μm	—	26	—
< 2 μm	—	2	—
Solubles (DOC)	—	1	—

with 11–17% of the total carbon. The percentage of total organic carbon extracted into the humic acid fraction is considerably higher and increases from mull (26%) to moder (50%) and mor (62%). Combined yields for the fulvic acid and humic acid fractions are rather low. Relative yields depend on the particle size of the organic matter, the type and concentration of extractant, and the ratio of extractant to organic matter. In the present investigation a constant extractant/organic matter ratio was used. Relative yields of the combined fractions fulvic acid and humic acid decrease from mor (73%) to moder (62%) and mull (43%). This is consistent with an increasing stabilization of organic matter in organo–mineral complexes. Simultaneously the percentage of carbon in the humin fraction decreases. The carbon distribution observed is due to the extraction with 0.5M NaOH, which leads to low amounts of humic acid extracted from calcareous soils, like the mull soil described here. Most of the organic matter in the acid, sandy A horizon of the mor humus is extractable by aqueous alkaline solutions, such as 0.5M NaOH.

Results from the density fractionations show that the percentage of total organic carbon bound in organo–mineral complexes decreases from mull (87%) to moder (44%) and mor (21%). The carbon in plant residues increases concurrently, indicative of a low degree of humification in the mor and a high degree of humification in the mull soil. Decomposition experiments with beech litter show that organo–mineral complex formation in forest soils is associated with the presence of earthworms (Ziegler and Zech, 1992a). The carbon distribution observed in the mull, moder and mor soils

TABLE 3

C/N ratios (and carbon concentrations, g kg^{-1}) in density and aggregate size fractions of mull, moder and mor A horizons

	Mull Ah	Moder Ah	Mor Aeh
<i>Density fractions</i>			
Plant residues	18 (370)	20 (370)	30 (460)
Organo-mineral complexes	12 (115)	18 (200)	20 (290)
<i>Aggregate-size fractions</i>			
Floatables	—	31 (343)	—
2000–200 μm	—	14 (5)	—
200–20 μm	—	15 (26)	—
20–2 μm	—	13 (41)	—
< 2 μm	—	11 (53)	—
Solubles (DOC)	—	5 (131)	—

emphasizes the close relationship between a high soil biological, in particular macrofaunal activity characteristic for the mull soil and organo-mineral complex formation.

The aggregate size fractionation was only carried out with the moder A horizon (Table 2). In this horizon only 3 and 1% of the carbon are present as floatable and water-soluble organic carbon, respectively; 2% of the carbon are bound in the clay fraction. Carbon in the macroaggregate fraction comprises 23% of the total. The microaggregate size fractions 200–20 μm (45%) and 20–2 μm (26%) play the major role in organic carbon storage.

Table 3 gives the C/N ratios of the density fractions of mull, moder and mor A horizons and of the aggregate size fractions of moder Ah. The C/N ratios increase from mull to moder and mor for the plant residues as well as for the organo-mineral complexes. In all samples the C/N ratios of the plant residue fraction are higher with values of 18–30 compared to the C/N ratios of the organo-mineral fractions with C/N ratios of 12–23, indicative of a higher degree of microbial modification of the organic matter in organo-mineral complexes. This finding is in good agreement with results of Turchenek and Oades (1979), Spycher et al. (1983) and Sollins et al. (1984). In the aggregate size fractions the C/N ratios also decrease from coarse (floatables: 31; 2000–200 μm : 14) to fine fractions (< 2 μm : 11), i.e., from macroorganic matter to organo-mineral associations.

Distribution of individual compound classes

Humification or microbial alteration of lignin is associated with ring cleavage and side-chain oxidation (Kirk and Farrell, 1987). The degree of microbial alteration of lignin can be characterized using the acid/aldehyde ratio of

the vanillyl units within the CuO oxidation products (Hedges et al., 1988). This value ranges from ~ 0.1 for many lignins found in plant litter to values higher than 1–3 in mineral soils. Concurrently, the yields of CuO oxidation products decrease with increasing degree of lignin alteration. (Ertel and Hedges, 1984; Kögel, 1986; Ziegler et al., 1986). The lignin-type material extractable in the fulvic and humic acid fraction of forest soils generally shows a higher acid/aldehyde ratio compared to the lignin in bulk soil organic matter. The more or less altered lignin is found in the humic acid fraction, whereas the unmodified lignin remains in the humin fraction (Table 4). The behaviour of lignin in the classical humus fractionation was investigated in a series of materials representing different stages of biodegradation, from fresh litter material and the organic matter of the forest floor horizons to the mineral A horizons. With increasing degree of lignin alteration an increasing amount of aromatic carbon is extractable in the humic acid fraction (Fig. 1).

A trend of lignin distribution is also observed in the density fractions (Table 4). Generally the degree of lignin side-chain alteration in organo–mineral complexes is higher compared to the respective plant residues. This is associated with lower yields of CuO oxidation products in the fraction of organo–mineral complexes (Table 4). The degree of lignin side-chain oxidation in organo–mineral complexes increases from mull to moder and mor, as indicated by acid/aldehyde ratios $(ac/al)_v$ of 0.38, 0.64 and 0.83, respectively. The lignin in the organo–mineral complexes of soils with a high macrofauna activity, like the mull soil investigated here, is only slightly modified, compared to the lignin in organo–mineral complexes of moder and mor soils. This is due to the fact that the alteration of lignin during the gut passage in earthworms is negligible (Ziegler and Zech, 1992b).

TABLE 4

Yields of CuO oxidation products (V+S+C, mg g⁻¹ org. matter) and acid/aldehyde ratios for the vanillyl unit of the CuO oxidation products in humic and density fractions in the A horizon of the three different forest soils

	$(ac/al)_v$			V+S+C		
	Mull Ah	Moder Ah	Mor Aeh	Mull Ah	Moder Ah	Mor Aeh
<i>Humic fractions</i>						
Fulvic	1.75	0.59	1.77			
Humic acid	0.67	0.80	1.63			
Humin	0.38	0.33	nd			
<i>Density fractions</i>						
Plant residues	0.21	0.53	0.38	18.70	17.06	10.54
Org.–min. complexes	0.38	0.64	0.83	8.74	9.90	7.69

nd = not detected.

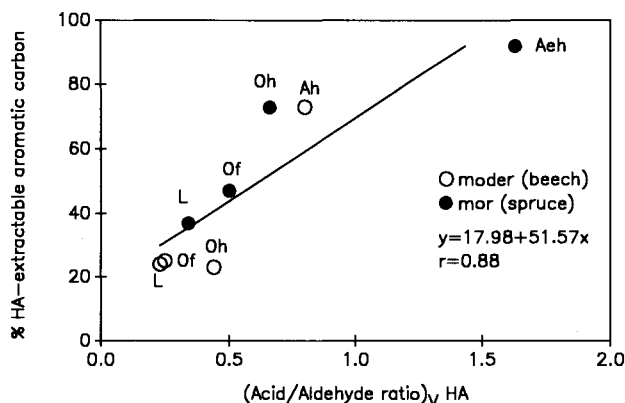


Fig. 1. Amount of aromatic carbon extracted in the humic acid fraction expressed as a percentage of the total aromatic carbon (both determined by CPMAS ^{13}C NMR spectroscopy) related to the degree of lignin oxidation as determined by the acid/aldehyde ratio of the vanillyl units of the CuO oxidation products (from Kögel-Knabner et al., 1988).

CONCLUSIONS

The investigation of humification processes in forest soils is possible via a combined approach to both the fractionation procedures and the chemical characterization of the fractions obtained. An examination of the data provided by the different methods reveals that the physical fractionation procedures reduce the complexity of the samples and allow a chemical characterization of the fractions, which represent compartments of soil organic matter associated with different stages of humification. The carbon concentrations in the aggregate size fractions increase significantly from the macro- to the microaggregates and to the soil clay fraction. Simultaneously a decrease of C/N ratios is observed, which emphasizes the strong influence from microorganisms on the finer structural units. The analysis of density fractionated samples shows that the light fraction is composed mainly of plant residues as far as the lignin data are concerned. The heavy fraction shows high amounts of strongly modified lignin. The classical humus fractionation produces fractions according to the solubility in alkaline aqueous solvents, generally not in line with the humification processes operating in A horizons of forest soils. However, the fractionation procedure provided useful data for the structural changes during humification of lignin. The humic acid fraction contains the aromatic carbon with the highest degree of structural modification. Thus the amount of aromatic carbon extractable with aqueous alkaline solvents is correlated with the degree of lignin modification during humification. Therefore, the classical humus fractionation into fulvic acid, humic acid and humin fractions seems suitable for the investigation of microbial alteration and humification processes of lignin.

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Phosphorus mineralization during laboratory incubation in soils derived from different textured parent materials

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ABSTRACT

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The dynamics of phosphorus in soils derived from different textured parent materials were studied. Soils weathered from the three parent materials were Haploborolls but different in texture and solum depth. Microbial phosphorus by hexanol fumigation, and mineralization rates of organic P (P_o) were assessed with a ^{32}P dilution technique and P was fractionated using a modification of the method of Hedley et al.

Microbial P and P_o mineralization rates were significantly affected by parent materials. The high rates of mineralization for the fine-textured soils correspond well with their higher content in substrates able to mineralize e.g. total carbon, P_o and microbial P_i . The residual P (e.g. more stable P_i and P_o forms) is the principal P fraction in the analyzed Mollisols.

INTRODUCTION

The amounts and forms of soil phosphorus (P) vary with soil-forming factors (Walker, 1965; Smeck, 1973). Moreover, Walker (1965) emphasized the role of P as perhaps the key element in pedogenesis because of its great ecological significance. More recently Cole and Heil (1981) have discussed the influence of P concentration on the tendency of C and N to accumulate during pedogenesis.

Precipitation inputs of P are generally below $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in both temperate (Likens et al., 1977) and tropical (López-Hernández, 1991) ecosys-

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tems. Therefore P, one of the macronutrients contained in soil organic matter, must be supplied almost entirely by the parent materials (Walker, 1965).

Phosphorus released in soils from weathering of primary P-bearing minerals and additions of plant residues and fertilizers combines primarily with the clay fraction (Black, 1968; López-Hernández and Burnham, 1974). As a result, the P percentage of the clay fraction usually exceeds that of the coarser particle size fraction. Moreover, if other conditions are similar, the P percentage of the soil as a whole usually increases as the texture becomes finer (Walker and Brown, 1936; O'Halloran et al., 1985, 1987).

The inorganic fractionation of P by Chang and Jackson (1957) and later modifications (Williams et al., 1967) has been used to characterize inorganic P (P_i) and has served as the basis for concepts of P transformation with pedogenesis (Westin and De Brito, 1969; Smeck, 1973). The method, however, does not differentiate organic phosphorus (P_o) fractions which are recognized to be very important in quantity and which play a significant role in plant nutrition. An alternative P fractionation method was developed by Hedley et al. (1982) to characterize not only P_i but also labile and more stable P_o in soils under different incubation conditions and management (Hedley et al., 1982; Tiessen et al., 1984; O'Halloran et al., 1987).

The availability and distribution of P have been studied across environmental gradients and as a function of topography (Roberts et al., 1985; López-Hernández, 1987). However, little detailed information exists on the distribution of organic and inorganic soil phosphorus as affected by different textured parent materials. Therefore, the primary purpose of this study was to determine the influence of different textured parent material on the nature and distribution of soil P by using a modified Hedley et al. (1982) sequential technique. A secondary objective was to relate P_o mineralization rates with the textural characteristics of the soils.

METHODS

Site description

Soils from the summit position of three toposequences were used in this study. The sites are located in southwestern North Dakota, and have been the subject of intensive studies on erosion, organic carbon and nitrogen changes resulting from cultivation (Aguilar, 1984; Schimel et al., 1985; Schimel, 1986). The toposequences were on three different parent materials with varying textures (sandstone, siltstone and shale) with grassland as the native plant component. The siltstone site has never been fertilized; the other sites have received about $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Schimel et al., 1985). The soil of all three sites were classified as Typic Haploborolls (Soil Survey Staff, 1975). Selected soil properties are shown in Table 1.

TABLE 1

Selected soil chemical and textural properties of the analyzed soils

Parent material	% Sand	% Silt	% Clay	% Carbon	% Nitrogen	Total phosphorus (mg kg ⁻¹)	Organic P (mg kg ⁻¹)
Sandstone	71	16	13	1.58	0.16	554	204
Siltstone	20	56	24	3.23	0.31	685	325
Shale	13	49	38	2.44	0.26	662	290

Field sampling

Soil samples for incubations were collected in August 1984. Three 7.5 cm diameter, 10 cm deep cores were taken from each site. The samples were kept in a cooler with ice and returned to Fort Collins, Colorado for analysis.

Incubations

Fifty grams < 2 mm samples of dry soil were placed into Nalgene PMP wide mouth 125 ml jars, wetted to 50% field capacity with distilled water and incubated with 1 ml of garden soil solution (E.T. Elliott, pers. commun., 1987) to restore biological activity. The wetter samples were left to incubate for 4 days. After that period, 2 ml ³²P-labelled 10⁻⁵M KH₂PO₄ carrier with an activity of approximately 40 μCi ml⁻¹ was added to the soil in order to obtain 80% of field capacity and the system was mixed thoroughly with a glass rod to give a distribution of ³²P in the soil as uniform as possible. The labelled soils were maintained in an incubation chamber designed to measure CO₂-C evolution and at a constant room temperature (25°C). Three aliquots from each sample were destructively sampled at days 0, 2, 7, 14, 21 and 51. On each sampling day the individual systems were thoroughly mixed to distribute the ³²P evenly and sub-samples were taken for subsequent phosphorus, activity and water contents analysis.

Isotope analysis

On each sampling date, triplicate samples from each site were taken in 100 ml centrifuge tubes and shaken for 1 h in a reciprocal shaker with 40 ml of de-ionized water plus 0.5 g of bagged anion exchange resin (Dowex 1 * 80-50 > 30 mesh) in a bicarbonate form.

After the shaking period the resin bag was removed and P_i was recovered by exchanging with 30 ml of a 0.5M HCl solution for 1 h. Aliquots of the resin recovered were analyzed for P_i by Murphy and Riley (1962) reagent and for ³²P_i by Cerenkov liquid scintillation counting. Isotopic exchangeable phos-

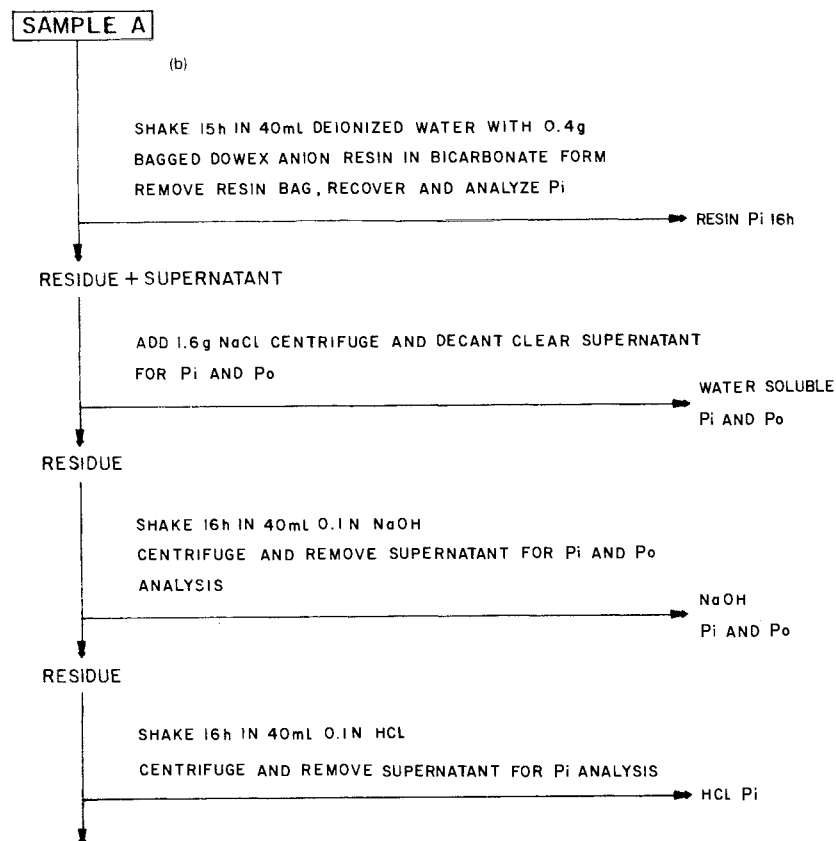
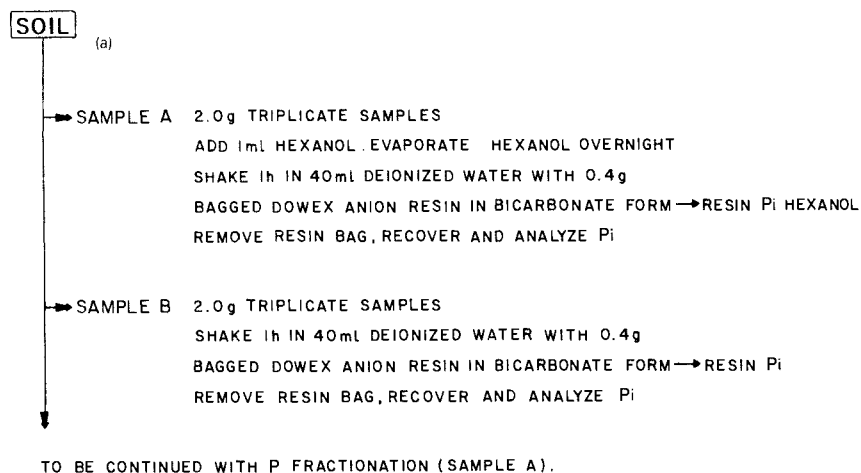


Fig. 1. (a) Microbial P_i determination. Microbial P_i corresponds to Hexanol-Resin P_i minus Resin P_i . (b) Modified Hedley et al. (1982) P fractionation method.

phorus (IEP) was determined after decay and humidity corrections at each sampling date by using the isotope dilution technique (White, 1976) according to the formula:

$$\text{IEP} = \frac{\text{total activity added (cpm g}^{-1} \text{ soil)}}{\text{specific activity of resin extract (cpm } \mu\text{g}^{-1} \text{ P)}}$$

Resins have been shown to adsorb practically only inorganic phosphorus (Hedley et al., 1982), therefore during the incubation period any increase in IEP is a direct consequence of the release of P_i by microbial activity, since exchange of ^{32}P with inorganic mineral forms appears to contribute little to IEP in the studied soils (Sweet, 1981).

Rates of P_o mineralization were calculated from the difference between IEP values at the start (0 day) and IEP values at a given incubation period.

Phosphorus fractionation

At the last sampling date (day 51) a modified version of the Hedley et al. (1982) fractionation of soil P was performed (Fig. 1). In the procedure, a sequential extraction removed labile P_i and P_o first, followed by the more stable forms. The most biologically labile P_i was extracted first with anion exchange resin (Amer et al., 1955). A duplicate sample of the soil was treated with hexanol (McLaughlin et al., 1986) and then extracted with the resin. The extra P_i and P_o extracted (Hexanol-Resin P minus Resin P) corresponds to part of the microbial P present in the soil (Fig. 1a). The suspension was flocculated with 1.6 g of NaCl, the clear solution contains mainly P_o desorbed from the soil surface.

The resin extraction was followed by sodium hydroxide to remove P_o associated with humic compounds (Batsula and Krivonosova, 1973; Fares et al., 1974; Bowman and Cole, 1978) and P_i more strongly bound to Fe and Al compounds (López-Hernández and Burnham, 1974; Burnham and López-Hernández, 1982). An acid extractant (1M HCl) removed mainly apatite-type minerals (Williams et al., 1971). Residual P was not analyzed, a separate total P analysis by NaOH fusion was performed, however.

Total P (P_t) in the neutralized extracts were determined by acidified sodium persulfate oxidation (Environmental Protection Agency, 1971) and P_o calculated as the difference between P_t and P_i .

RESULTS

Rates of mineralization

During the experimental incubation period, the amount of isotopic-exchangeable phosphorus (IEP) increased (Fig. 2). At the beginning of the ex-

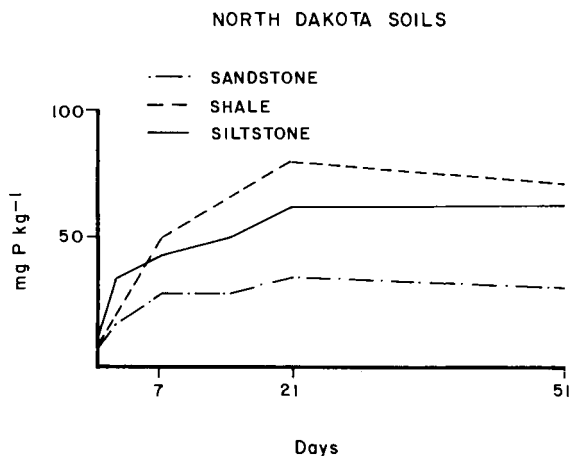


Fig. 2. Changes in isotope exchangeable phosphorus (IEP) during incubation.

periment (days 0–7), the mineralization increased rapidly, particularly in the case of the shale and siltstone sites, and in a minor extent for the sandstone. Then the slopes of the curves decrease (days 7–21), and finally values become constant until the end of the incubation study (days 21–51). A 14 day period seems to be enough for an important part of the mineralization process to occur for the sandstone and siltstone, whereas the shale site still showed a significant increase in IEP values after 14 days of incubation. Assuming an incubation period of 51 days a low rate of mineralization was found for the sandstone ($0.48 \text{ mg P kg}^{-1} \text{ day}^{-1}$, Table 3) when compared with the shale and siltstone with 1.25 and $1.03 \text{ mg P kg}^{-1} \text{ day}^{-1}$ mineralized, respectively. Decay corrected specific activities (SA, data not presented) showed a decrease during the incubation period that is a consequence of a flush of $^{31}\text{P}_i$ released by mineralization.

P fractionation

The shale parent material site gave the highest value in resin P_i ($7.1 \mu\text{g g}^{-1}$) as compared with the other two sites (3.9 and 4.5 for sandstone and siltstone, respectively) (Table 2). Microbial P_i was also present in appreciable amounts in the fine materials (14.3 and $10.3 \mu\text{g g}^{-1}$ for the siltstone and shale, respectively), whereas the coarse-textured sandstone site yielded a lower value ($7.3 \mu\text{g g}^{-1}$). As expected after resin extraction the amount of P_i remaining in solution was low (0.63 – $0.80 \mu\text{g g}^{-1}$) in all the three sites. On the other hand, P_o remaining in solution was, however, important although the values did not differ significantly between sites (Table 2). Inorganic P extracted with NaOH was also significantly higher in the siltstone than in the sandstone and shale.

TABLE 2

Phosphorus fractions (mg kg^{-1}) from the sequential extraction. Standard deviation in parentheses

Parent material	Resin P_i 16 h	Microbial P_i	P_i water	P_o water
Sandstone	3.9 (0.31)	7.6 (1.38)	0.63 (0.05)	9.3 (1.10)
Siltstone	4.5 (1.90)	14.3 (1.6)	0.80 (0.19)	10.8 (0.95)
Shale	7.1 (3.15)	10.3 (6.8)	0.66 (0.19)	9.7 (0.54)
Parent material	NaOH- P_i	NaOH- P_o	HCl- P_i	Sum fraction
Sandstone	15.5 (2.87)	44.0 (7.50)	159.1 (12.60)	240.03
Siltstone	20.2 (1.80)	56.0 (1.80)	139.6 (15.98)	246.20
Shale	12.1 (0.69)	43.4 (13.33)	164.9 (44.00)	248.16

A similar result was found for NaOH extracted P_o . HCl-extracted P_i was high for the sandstone and shale sites, and significantly differs from the siltstone site.

Finally, total P as determined by NaOH fusion as well organic P for the fine-textured parent material (shale and siltstone) exceeded the value of the coarse particle-size sandstone (Table 1). Subtracting the sum of the fractions from total P, residual P is calculated. It appears that residual P which corresponds to the more stable P_i and P_o forms, is the principal P fraction in all the analyzed Mollisols; residual P fraction was higher in the shale ($414 \mu\text{g g}^{-1}$) when compared with the sandstone and siltstone with 314 and $359 \mu\text{g g}^{-1}$, respectively.

DISCUSSION

The productivity of many natural and agricultural ecosystems is determined by the quantity of P_i made available through mineralization during the growing season. In some agricultural ecosystems, P fertilizer additions are non-existent or negligible, particularly in the case of many Mollisols in the Great Plains, where Haas et al. (1961) measured a significant average reduction in P_o of 35% after 31 to 48 years of cropping, compared with virgin sod. In this situation, the quantities of P_i coming from mineralization are of paramount importance. Most plant species have low P requirements (Fox, 1981; López-Hernández et al., 1987). Consequently, only a small proportion of the P_o must be mineralized to provide P_i requirements.

Higher rates of mineralization were measured in the case of the fine-textured parent material soils (1.25 and $1.03 \text{ mg P kg}^{-1} \text{ day}^{-1}$, Table 3) compared with the sandstone derived soil ($0.48 \text{ mg P kg}^{-1} \text{ day}^{-1}$). These values

TABLE 3

IEP values at the beginning (T_0) and 51 days of the incubation period. % P_o mineralized at 14 days as compared with 51 days

Parent material	IEP at T_0 (mg P kg ⁻¹)	IEP at 51 days (mg P kg ⁻¹)	mg P kg ⁻¹ mineralized	Rate of mineralization (mg P kg ⁻¹ day ⁻¹)	% IEP 14 days/51 days
Sandstone	5.9	30.3	24.4	0.48	93
Siltstone	9.9	62.2	52.3	1.03	79
Shale	6.7	70.3	63.6	1.25	66

are higher than the theoretical value (0.13 mg kg⁻¹ day⁻¹) found by Harrison (1982a) for a woodland in the Lake District in England. The data, however, cannot be compared directly because the method used by Harrison (1982b) included a shorter incubation period. Harrison's experiments were performed using an incubation temperature of 13°C, the experiments reported here were done at a higher temperature (25°C) and perhaps a more optimal moisture content. Temperature is very well known to affect the rate of P_o mineralization (Thompson and Black, 1947; Ruiz and López-Hernández, 1977).

The high rate of mineralization for the fine-textured soils corresponds well with their higher content in substrates able to mineralize e.g. total carbon, organic P and microbial P_i (Tables 1 and 2).

O'Halloran et al. (1985, 1987) studied P fractionation in a spatial variability design study. They reported a decrease in resin P_i and NaOH P_i with increasing sand content. The results presented here are not entirely comparable with O'Halloran's results because of the reduced number of samples used. The information confirms, however, the results in the case of resin P_i , but not for NaOH extractable P_i . When comparing the information obtained for HCl- P_i , non significant differences were found among the different textured parent materials. O'Halloran et al. (1985), on the contrary, reported a positive correlation for HCl- P_i and sand content.

Schimel (1986) measured net gross N mineralization and CO₂ evolution during the first 4 days of incubation in soils derived from the same parent materials. He concluded that net N mineralization was similar in all sites; however, the gross N mineralization, and particularly the CO₂ evolution, was higher in the fine-textured materials than in the coarse-textured sandstone. The results presented by Schimel (1986) for N and C coincide with the information presented here for P_o mineralization rates. CO₂ evolution curves (to be discussed in another paper) during a 28 d incubation period resembles those obtained for isotopic exchangeable P, indicating that the increase in the exchangeable P fraction (IEP) is a direct consequence of the release of P_i by microbial activity.

CONCLUSIONS

Our results for soils with the same taxonomic classification (Typic Haploborolls) but differing in textural characteristics show that higher rates of mineralization occurred in the fine-textured parent material soils compared with the sandstone derived soil. The high rate of mineralization for the fine-textured soils corresponds well with their higher content in organic P and microbial P_i . The residual P (e.g. stable P_i and P_o forms) is the principal P fraction in the analyzed Haploborolls.

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An improved sieving machine for estimation of soil aggregate stability (SAS)

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ABSTRACT

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According to the method for estimating soil aggregate stability (SAS) described by Kemper and Koch, a new sieving device has been constructed to improve manipulation and the rapidity of the analysis. The method of determination is described. Multiple replications of several samples that have been carried out by the members of the Austrian Soil Physics Research Group, showed a small standard deviation on the same soil samples. Storage of soil samples before measuring may cause a variation (increase) in SAS. The spatial variability of SAS on three field plots turned out to be relatively low. However, statistically significant differences were obtained, which proofs the high precision of the modified machine. Furthermore a significant influence of cattle slurry treatments on SAS of permanent grassland soil could be detected.

INTRODUCTION

Soil aggregate stability (SAS) is affected by various biotical and abiotical factors like microbiological activity and/or clay content as well as by the land-use practices. The concept of aggregate stability reflects many soil structural parameters, but as a result, it is a function of whether the cohesive forces between particles resist the applied disruptive force of water.

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Different methods of measuring soil aggregation focus on size distribution and stability of soil aggregates (Jastrow and Miller, 1991). Basic methods have been employed by Yoder (1936), Kemper and Chepil (1965), Williams et al. (1966) and Kemper and Rosenau (1986). The purpose of the aggregate stability measurements is to answer questions related to the effects of soil management and wind erosion (Kemper and Chepil, 1965; Kemper and Rosenau, 1986). Single- and multiple-sieve methods are equally well correlated with field phenomena of practical importance (Kemper and Rosenau, 1986). However, the measurement of aggregate stability by the single-sieve method is simpler and more rapid; more complex methods are scarcely justified (Marshall and Holmes, 1988). Therefore, the single-sieve method of Kemper and Koch (1966) was modified, improved and tested by the Austrian Soil Physics Research Group. The determination of soil aggregate stability according to Kemper and Koch (1966) gave good response, also in other application fields of soil physics (Johnston et al., 1942; Russel, 1971; Elwell, 1986; Frohner, 1991).

METHODS

To determine SAS, a method of wetting soil by immersion into salt-free water has been used. The newly designed sieving apparatus is made of a metal tub (stainless steel) containing a tipping device, the electric drive and the transformer (Figs. 1 and 2).

A three cup unit can be hooked to the seesaw at each side. The sieve holder,

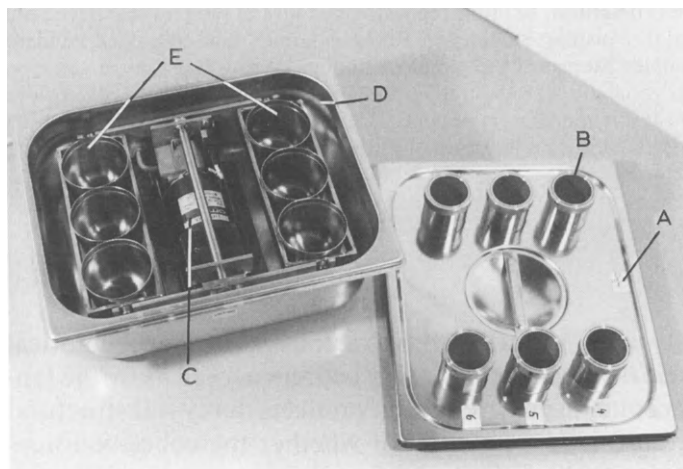


Fig. 1. General set up of the modified wet sieving machine*. A=sieve holder; B=sieve; C=motor and transformer (220 V into 90 V); D=bath; E=rocker plate with 6 cans, containing 70–90 ml aqua dest. *Name of manufacturer available on request!

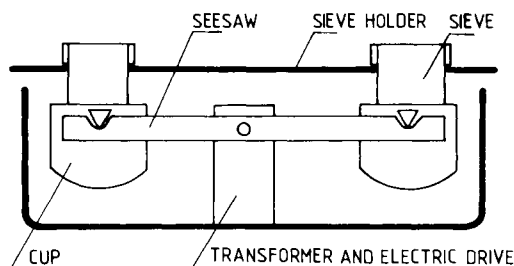


Fig. 2. Cross section of the improved sieving machine.

designed for 6 sieves that project into the cups, can be placed on top of the tub. The sieves are stainless steel screens and, according to the original method, are 38 mm in diameter with a wire diameter of 0.165 mm and a hole size of 0.25 mm square. The machine provides a mechanical stroke length of 12.7 mm to the sieves at a frequency of 42 cycles/min. In the original device described by Kemper and Rosenau (1986) the sieves are moved up and down. In the presented sieving machine the cups are in motion. This reduces the size of the machine, improves the manipulation and minimizes the expenditure of energy and the abrasion of the electric drive. Moreover, the alternative use of several sieve holders allows rapid measurements of up to 125 samples a day.

According to Kemper and Rosenau (1986), results are more reproducible if aggregates of a limited size range are selected. Aggregates of 1 to 2 mm in diameter were found to be best suited to the screen size and sieving speed employed in this procedure. A good correlation between this one-sieve method and results using several size ranges of aggregates and sieves were found by Bryant et al. (1948), Strickling (1951), Schaller and Stockinger (1953) and Panabokke and Quirk (1957).

Reagent: 0.1 mol solution of $\text{Na}_4\text{P}_2\text{O}_7 \cdot \text{H}_2\text{O}$

Procedure: 4 g of air-dried soil aggregates (1 to 2 mm in diameter) are put onto the sieve. The immersion of the soil aggregates in distilled water is followed by five minutes of wet sieving, leaving the sample immersed during movement. The remaining aggregates are oven dried (105°C) and weighed (*A*). Then the aggregates are destroyed by 0.1 mol $\text{Na}_4\text{P}_2\text{O}_7$ solution, leaving only the sand fraction > 0.25 mm on the sieve. The sand is oven dried and weighed (*B*). If the relative humidity of the laboratory is above 30%, an additional 4 g of aggregates should be oven dried to determine the moisture content of the sample. The soil aggregate stability (SAS) is calculated as follows:

$$\text{SAS}(\%) = \{ [A(\text{g}) - B(\text{g})] / 4 - B(\text{g}) \} \times 100$$

A first laboratory intercomparison was a pilot test of the improved sieving machine. Nine laboratories conducted SAS measurements on a Eutric Cambisol from Lower Austria (soil 1, Table 1) with several repetitions between May and October 1991.

This first test was followed by a second intercomparison in December 1991 with 3 soils (Table 1) and a short time limit for SAS determination of 3 weeks.

The test soil varied significantly in the lime and organic matter content, pH and particle size distribution. The statistical analysis was carried out according to the Student–Newman–Keuls, Duncan's test.

In addition to the laboratory intercomparisons two case study were conducted in order to confirm the suitability of the chosen aggregate fraction of 1 to 2 mm for the description of different soil conditions. The first case study to be presented was conducted in a typical agricultural area of Lower Austria. Two plots were chosen from fields with crop rotation, one plot from a permanent grassland. The soil type was classified as a Calcic Chernozem (ISSS, 1986). Results of soil characterization can be found elsewhere (Gerzabek et al., 1992). Nine samples (0–20 cm) were taken from each plot (10 times 10 m) every five meters in a square in July 1991. The samples were analysed with three replicates.

The second case study deals with the effect of the increasing amounts of cattle slurry, spread on permanent grassland. Equivalents of 0, 96 and 480 kg N ha⁻¹ yr⁻¹ were applied on 4 times 5 m plots in four replications since 1978. In September 1991 samples from different soil depths (0–5, 5–10 and 10–20 cm) were taken for SAS measurements and analysed with three replications.

TABLE 1

Soil characteristics of test samples

Soil	Location	Soil type	Particle size distribution (%)			Organic matter content (%)	Lime content (%)	pH (CaCl ₂)
			Clay	Silt	Sand			
1	Lagerfeld, Wieselburg, Lower Austria	Eutric Cambisol	20	58	22	2.0	6.1	7.1
2	Schloßpark, Petzenkirchen, Lower Austria	Eutric Cambisol	26	71	3	2.5	0	6.8
3	Niederstuttern, Trautenfels, Styria	Eutric Cambisol	11	68	21	4.7	6.3	7.5
4	Spatenpflug, Feldbach, Styria	Dystric Fluvisol	19	46	35	2.5	0	6.1

RESULTS AND DISCUSSION

The soil used for the pilot test showed a SAS of 29% and a total standard deviation of 5.5% (Table 2). The mean standard deviations of the nine laboratories reached only 4.0% which seems to be a quite good value for a pilot test. The evaluation of the obtained results led to the conclusion that SAS increases with the storage time of the sample (Fig. 3). The linear correlation was statistically significant.

This effect has already been observed by Kemper and Koch (1966) and it may be due to lasting microbial activity causing further agglomeration even in air-dried soil samples (Orchard and Cook, 1983).

The three test samples of the second laboratory intercomparison varied distinctly in their SAS (Table 2). Soils 2 and 3, originating from permanent grassland, expressed a higher SAS as compared to soil 4 from a maize field without crop rotation. As can be observed for various methods of soil analysis, decreasing SAS values led to higher standard deviations. The mean average laboratories standard deviation of soils 2, 3 and 4 reached 1.8%, 1.0% and 2.7%, respectively. Only four mean values were significantly different, which proofs the high practicability and reproducibility of the discussed method.

Table 3 shows the results of SAS measurements on three field plots. The two sites with crop rotation exhibited significantly lower soil aggregate stability as compared to the permanent grassland. This well known effect is due to the high organic matter content of the grassland (Kemper and Koch, 1966). The spatial variability of SAS was relatively low. However, statistically signif-

TABLE 2

Results of the first (soil 1) and second (soil 2-4) laboratory intercomparison (SAS)

No. of laboratory	Soil 1		Soil 2		Soil 3		Soil 4	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
1	27	4.0	60	2.5	86*	3.3	27	1.0
2	23*	2.9	62	4.2	93	1.3	39	7.4
3	26	0.6	58	0.8	93	0.6	33	1.7
4	31	3.8	65	1.2	94	0.4	39	2.4
5	35*	4.1	60	1.2	94	0.5	30	1.5
6	29	10.1	65	2.7	93	0.4	34	2.4
7	36*	3.5	63	1.5	93	1.9	49*	2.8
8	26	0.3	66	1.1	97	0.5	39	4.4
9	30	5.7	57*	1.5	92	1.3	30	0.6
10	—	—	58	2.5	91	0.3	52*	1.7
11	—	—	66	0.9	94	0.4	38	3.3
All	29	5.5	62	3.7	93	2.2	37	7.7

Mean values with * are significantly different at the 5% level.

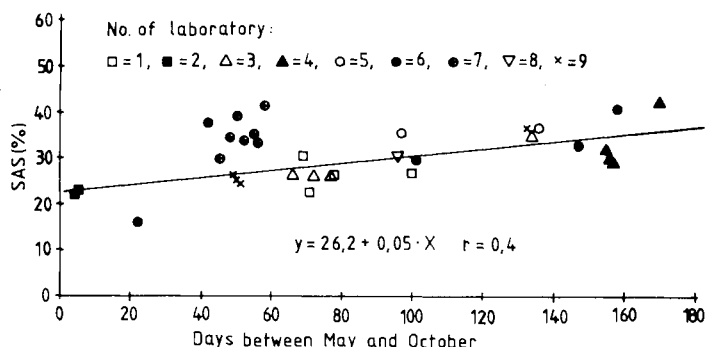


Fig. 3. Response of soil aggregate stability (wt%) of a test-soil sample to storage time.

TABLE 3

Response of soil aggregate stability (wt%) on 10 times 10 m plots to differences in agricultural land use of a Calcic Chernozem in Lower Austria ($n = 3$)

		Coordinates of the sample (m)		
		0	5	10
Crop rotation (barley)	0	22.3 bc	21.8 c	21.0 cd
	5	19.0 e	24.3 a	20.3 de
	10	23.3 ab	21.4 cd	23.8 a
Crop rotation (corn)	0	32.2 c	36.6 a	27.3 d
	5	31.5 c	34.7 b	31.7 c
	10	25.7 e	34.5 b	31.0 c
Permanent grassland	0	93.8 c	94.4 bc	95.7 a
	5	95.1 ab	90.8 d	93.9 bc
	10	91.5 d	89.0 e	95.5 a

Mean values with different letters (a, b, c, d, e) are significantly different at the 5% level (Waller-Duncan's k -ratio t -test).

icant differences were obtained between the different samples of each plot, the lowest significant differences (5% level) being as low as 0.93%, 1.29% and 1.37% for the grassland, corn and barley plot, respectively. The mean standard deviation relative to the SAS mean values decreased from low SAS (barley: 2.65%, corn: 1.56%) to high SAS (grassland: 0.31%). The obtained values prove the high precision of the described sieving machine, which is therefore suitable to detect rather small differences in SAS.

Table 4 shows the results of the slurry treatment trial on permanent grassland. Generally, SAS decreased with soil depth, which may be explained by differences in root density and microbial activity (Alexander, 1977). Moreover, SAS responded to the cattle slurry treatments. Even the low slurry treat-

TABLE 4

Response of soil aggregate stability (wt%) of a Eutric Cambisol (soil 2, Table 1) under permanent grassland to increasing amounts of cattle slurry ($n=3$)

Treatment (kg N/ha ⁻¹ yr ⁻¹)	Soil depth 0–5 cm		Soil depth 5–10 cm		Soil depth 10–20 cm	
	mean	S.D.	mean	S.D.	mean	S.D.
0	77 a	7.3	71 a	8.5	58 a	7.4
96	78 a	13.3	62 a	7.6	63 a	4.7
480	67 a	5.5	57 ab	5.2	49 b	6.9

Mean values with different letters (a, b, c, d, e) are significantly different at the 5% level (Waller-Duncan's k -ratio t -test).

ment caused a significant decrease of SAS in the 5 to 10 cm layer. The high 480 kg ha⁻¹ yr⁻¹ treatment showed an more pronounced effect. In contrast, organic fertilization increased microbial activities and furthermore aggregate stability of arable plots (Christensen, 1986). Our divergent results may be caused by the decrease of root density, root length and root surface with increasing nitrogen supply of the permanent grassland (Sobotik and Eder, 1991). The importance of vegetation and soil micro-organisms to maintain high aggregate stability was confirmed by results of Tisdall and Oades (1980, 1982), Miller and Jastrow (1990) and Kandeler and Murer (1993).

CONCLUSIONS

The Austrian Physics Research Group modified the Kemper and Koch machine using the single size range of aggregates (1–2 mm) according to Kemper and Rosenau (1986). The measurement of aggregate stability (SAS) by this method is simple and rapid.

Multiple measurements conducted with a modified machine for the determination of soil aggregate stability (SAS) by the members of the Austrian Soil Physics Research Group during laboratories intercomparisons showed a small standard deviation on four test-soil samples.

The storage time of soil samples may cause a variation (increase) of soil aggregate stability.

The modified sieving machine is suitable to detect rather small differences in SAS, which was proved by a field experiment on the spatial variability. Furthermore, a significant influence of cattle slurry treatments on SAS of permanent grassland soil could be detected.

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Direct measurements of oxygen microprofiles and distribution of phospholipid-P in a two-phase soil–manure system

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ABSTRACT

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Concentrations of phospholipid-P (Plip-P) were used as an estimate of biomass in soil. Plip-P was correlated with biomass-C, as determined by chloroform fumigation–direct extraction, in soil from a fertilizer experiment, although a background of non-biomass Plip-P was suggested. The distribution of Plip-P was followed during three weeks in a two-phase model system designed to study microbial processes in and around a manure-saturated zone. Concentrations of Plip-P in the soil were unaffected by the presence of manure at ≥ 4 mm distance from the soil–manure interface. A sharp gradient between manure and soil phases was maintained throughout the three weeks. Oxygen microelectrodes (i.d. ca. 5 μm , o.d. 150–200 μm) were used for direct measurements of oxygen penetration into the manure-saturated zone. No decrease in oxygen concentration was recorded in the soil phase. By Day 1 oxygen penetrated only 0.15 mm into the manure, increasing to 2–2.5 mm after 21 days. During this period the diffusive flux of oxygen decreased five-fold. Aerobic decomposition of dissolved C in the manure was apparently restricted to a thin layer of 0–2 mm thickness below the soil–manure interface.

INTRODUCTION

The heterogeneity characterizing soil on a mm scale affects the distribution of oxygen. Wet aggregates may have an anaerobic centre, as demonstrated by direct measurements with an oxygen microelectrode (Sexstone et al., 1985). Oxygen depletion can also occur in organic “hot spots”, such as particles of fresh plant debris, as a result of intense mineralization activity (Parkin, 1987).

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Amendment of liquid manure to soil adds to the structural heterogeneity, because complete mixing of manure and soil is not possible. Anaerobic volumes will be formed as particulate organic matter in the manure effectively retains water, while the degradation of dissolved organic compounds ensures a rapid consumption of oxygen near the interface between the manure-saturated zone and the undisturbed soil. The development of high potentials for nitrification and denitrification within 0–5 mm distance above and below this interface, respectively, suggested that oxygen did not penetrate far into the manure for at least two weeks (Petersen et al., 1991a). Also, evidence for a high concentration of organisms within 2 mm distance from the interface was obtained from extractions of lipid-bound phosphate, which has been proposed as an index of living biomass (White et al., 1979). These observations indicate that there is an intense aerobic heterotrophic activity near the manure–soil interface where dissolved C and oxygen meet, and underneath this zone an anaerobic volume where processes like denitrification can occur.

More information is required about the correspondence between the phospholipid assay and other measures of biomass in soil. We first compared concentrations of lipid-bound phosphate with biomass-C measurements based on chloroform fumigation followed by direct extraction (CFDE). In the main experiment, profiles of phospholipid-P (Plip-P) were measured over a three week period in a two-phase soil–manure system. In addition to the Plip-P profiles the distribution of aerobic heterotrophic activity was examined by microelectrode measurements of oxygen penetration into the manure-saturated zone.

MATERIALS AND METHODS

Experiment 1

Biomass-C and phospholipid-P was measured in sandy loam soil (see Table 1) from a long-term fertilizer experiment at Askov, Denmark, initiated in 1894. Three plots representing the following treatments were selected: No N, Inorganic N (1 N), and Liquid manure (1 N); 1 N corresponded to 100 kg N ha⁻¹ for winter cereals. Sampling took place in late September, four weeks after harvest of the winter cereal. Five cores were taken randomly to 20 cm depth and sectioned in 5 cm depth intervals. Each depth interval was pooled and sieved (<5 mm), and stored in the dark at in situ temperature (12°C) for ca. one month before analysis. The water content of the 12 samples varied between 31 and 43% of WHC.

Biomass-C was determined in triplicate by the procedure described by Vance et al. (1987), except that 20 g fresh wt. soil and 40 ml extractant was used, and extracts were filtered through Whatman GF/F filters. The extracts were kept at 4°C until analyzed. Soluble C in fumigated and non-fumigated soil

TABLE 1

Some characteristics of the soils used for Experiments 1 and 2, respectively

	Expt. 1	Expt. 2
<i>Texture</i>		
Sand	77.6	85.0
Silt	11.8	9.5
Clay	10.6	5.5
<i>% C/%N</i>		1.2/0.15
No N	1.26/0.101	
Inorganic N	1.43/0.117	
Liquid manure	1.38/0.120	
<i>pH (KCl)</i>		5.1
No N	7.15	
Inorganic N	7.05	
Liquid manure	6.80	

samples was determined within 48 h of extraction on a Model 700 Total Organic Carbon Analyzer [O.I. Corporation; College St., Texas]. A 3 ml subsample was mixed with 3 ml 10% H₃PO₄ to dissolve CaSO₄. The conversion factor of 2.22 suggested by Wu et al. (1990) was used to calculate biomass-C.

The extraction of Plip-P was a modified Bligh-Dyer procedure (Petersen et al., 1991b). Approximately 2 g fresh wt. soil (triple determination) was extracted in 5 ml methanol and 2.5 ml dichloromethane (DCM) for at least 2 hours. Then 2.5 ml DCM and 10 ml NaBr solution (0.8 g ml⁻¹) was added and the phases allowed to separate for 12 hours. After centrifugation, a one ml subsample of the DCM phase containing the lipids was transferred to glass ampoules and evaporated to dryness under a stream of nitrogen in a fume hood. Two ml 185 mM K₂S₂O₈ was added and the ampoules heat sealed. After oxidation at approximately 100°C overnight, the liberated phosphate was determined according to Van Veldhoven and Mannaerts (1987).

Experiment 2

Soil (see Table 1) and liquid cattle manure for the second experiment was collected one month before the experiment was started. The soil was sieved (<2 mm) and both soil and manure stored at 4°C in the dark. Three days prior to Day 0 the water content of the soil was adjusted to field capacity, and then the soil was packed in cores of polyacrylamide (i.d. 44 mm), one cm at a time, to a height of 7 cm and with a density of 1.25 g cm⁻³. The cores were then preincubated at 20°C.

The liquid manure was sieved (<1 mm) immediately before use. This

fraction contained 8% dry matter, $644 \pm 7 \text{ mmol kg}^{-1}$ dissolved organic carbon and $142 \pm 20 \text{ mmol kg}^{-1} \text{ NH}_4^+$, while no NO_2^- or NO_3^- could be detected; the phospholipid concentration was $437 \pm 8 \text{ } \mu\text{mol kg}^{-1}$. The manure-saturated zone of the model system consisted of manure, air-dried soil, and a 3% silica gel prepared by ion exchange (Petersen et al., 1991a) in the proportions 3:4:3 by volume. This "manure phase" was allowed to solidify in rings of the appropriate diameter resting on a petri dish. Each sample was completed by placing a manure phase, cut to a thickness of 16 mm with a sharp knife, in the middle of a soil core (Fig. 1). Each sample was incubated at 20°C in 425 cm^3 PVC containers covered with parafilm; the sample rested on a nylon mesh allowing aeration of the sample from below.

At each sampling duplicate cores were sectioned in a number of depth intervals. The distribution of water and dry matter (after drying at 105°C for 24 hours) was examined after 1 and 21 days. From previous experiments it was known that any redistribution of water and solutes was symmetrical around the central layer under these experimental conditions (i.e. no gravitational flow). For the Plip-P analysis corresponding depth intervals above and below the centre were therefore pooled and subsampled, and results are presented as half profiles. Sampling took place after 1, 7, 14 and 21 days.

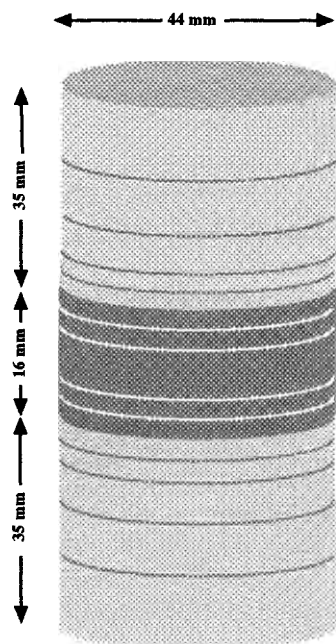


Fig. 1. The two-phase model system with a gel-stabilized mixture of soil and liquid manure placed between two pre-packed soil cores. The lines of sectioning are indicated (for depth intervals see Fig. 3).

A microelectrode was used to measure oxygen profiles in the manure phase. The construction and use of the electrode was described by Sexstone et al. (1985), except that in the present experiment the electrodes were equipped with a guard cathode (Revsbech, 1989), and the micro-manipulator used to insert the electrode was operated via a computer-controlled motor. As a sandy loam soil was investigated, it was necessary to further reinforce the electrode tip (i.d. ca. 5 μm ; o.d. 150–200 μm).

Oxygen microprofiles were always measured at the bottom side of the manure phase, because this interface (originally facing the petri dish during solidification) was more smooth. Control measurements at the top side showed that the oxygen profiles here were more variable, but that the penetration depth was of similar magnitude. The oxygen concentration of the soil atmosphere was at air-saturation right above the interface (cf. Fig. 5A); for practical reasons the soil phase was therefore completely removed prior to oxygen profile measurements. This did not affect the penetration of oxygen during the time it took to measure 5–10 profiles in each of the two replicates. Oxygen penetration was analyzed after 1, 4, 7, 14 and 21 days.

The flux of oxygen, J , into the manure phase was calculated for selected profiles using Fick's 1st law of diffusion:

$$J = \phi \times D_s \times \partial C / \partial x$$

where ϕ is porosity, $\partial C / \partial x$ is the concentration gradient between soil and manure phase, and D_s is the effective diffusion coefficient in the porous medium. A value of 0.50 was calculated for ϕ . $\partial C / \partial x$ was estimated from the slope of the individual profiles immediately below the interface. D_s was estimated from the diffusion of oxygen in pure water ($2.18 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) using the Millington–Quirk tortuosity relation (Jury et al., 1991):

$$\xi_l(\theta) = \theta^{(10/3)} / \phi^2$$

where ξ_l is the liquid tortuosity factor and θ is the volumetric water content (=porosity in the saturated zone). This gave a D_s of $8.65 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, which is close to the value of $8.5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ measured by Sexstone et al. (1985) in saturated aggregates of a silt loam soil.

RESULTS AND DISCUSSION

Experiment 1

Biomass-C, as determined by CFDE, was very constant with depth (Fig. 2A). Values for soil from the treatments "No N" and "Inorganic N" were almost identical, while biomass-C in the treatment "Liquid manure" was considerably higher. Average values for 0–20 cm depth were ($\mu\text{g C g}^{-1}$ dry wt. soil): 145 ± 12 , 145 ± 17 , and 260 ± 10 ; these values were not significantly

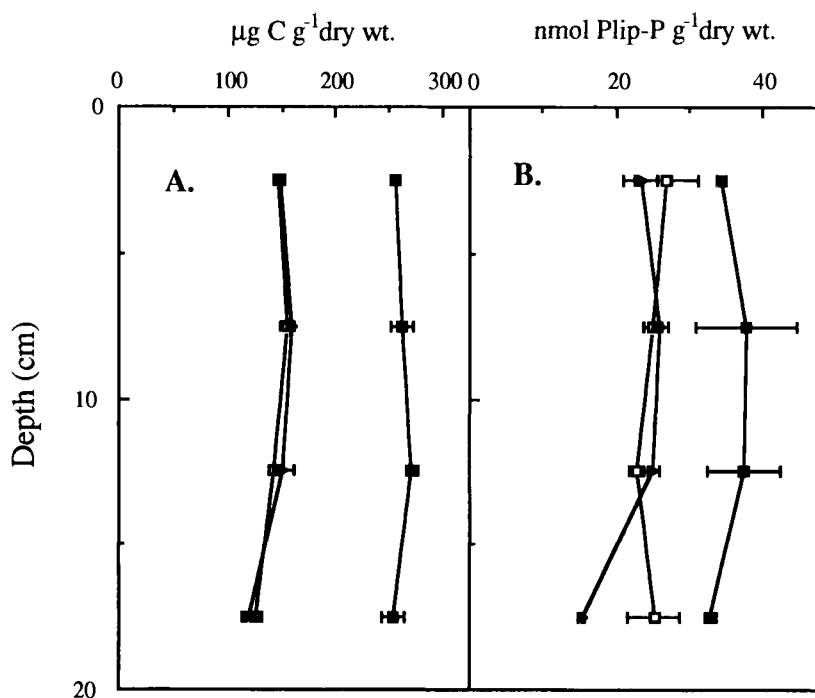


Fig. 2. Concentrations in soil of (A) biomass-C, determined by CFDE, and (B) phospholipid-P in three treatments from a long-term field experiment ($n=3$). Symbols: ($\square$) No N; ($\blacktriangle$) Inorganic N; ($\blacksquare$) Liquid manure.

different from values previously determined in the same treatments using the chloroform fumigation-incubation method (Petersen et al., 1991b).

Concentrations of Plip-P (Fig. 2B) were also relatively uniform with depth, except for a significant drop in the bottom layer of the "Inorganic N" treatment. Again, treatments "No N" and "Inorganic N" were at the same level, while the concentrations in the "Liquid manure" treatment were somewhat higher.

A plot of mean values of Plip-P vs. biomass-C for the individual samples gives the following regression line: $\text{Plip-P (nmol g}^{-1}\text{)} = 8.63 (\pm 2.44) + 0.105 (\pm 0.013) \times \text{biomass-C } (\mu\text{g g}^{-1})$, $r^2=0.87$, $P<0.001$. The slope of the regression line corresponds to approximately $100 \mu\text{mol Plip-P g}^{-1} \text{C}$, which is in accordance with earlier observations (Petersen et al., 1991b). Conversion factors determined for aerobic soil enrichment cultures and pure cultures of selected soil bacteria and fungi also suggest an average value in this range (J. Brinch-Iversen, pers. commun.). Since the intercept is significantly above 0, it suggests that there could be a background of lipid-bound P not associated with biomass (assuming that the CFDE procedure is a reliable measure of biomass). A potential source of non-biomass Plip-P is water-insoluble humic

acids that appear to be able to bind hydrophobic molecules firmly (Khan and Schnitzer, 1972). Kowalenko and McKercher (1971) determined Plip-P in a range of soils by the Bligh-Dyer method and two other methods. Based on a theoretical calculation they estimated that 10–20% of the Plip-P was associated with living biomass; their conversion factor corresponded to 65 $\mu\text{mol Plip-P g}^{-1} \text{C}$, which is low compared to most published values (see Brinch-Iversen and King, 1990 for references). In the present experiment Plip-P was correlated with biomass-C, and the level of Plip-P was 20–40 nmol g^{-1} dry wt. soil compared to a background of 5–10 nmol g^{-1} dry wt., suggesting that at least 50% of the Plip-P was associated with living organisms in the soil used for this experiment.

The possibility of a background pool of Plip-P questions the use of this assay for biomass measurements in environmental samples. On the other hand, if such a “stabilized” pool could be quantified by calibration against other measures of biomass, the method may still be useful for studying biomass dynamics in space and time, as exemplified in Experiment 2. A field study of biomass determinations involving several soil types and treatment effects is in preparation.

Experiment 2

This experiment compared the distribution of Plip-P with the distribution of aerobic heterotrophic activity in a two-phase soil-manure system during a three week period.

The distribution of water and dry matter is shown in Figs. 3A and 3B, respectively; except for a slight compaction there were no difference between the results for Day 1 and Day 21, and only the latter are shown. There was a strong retention of water within the manure phase throughout the three week period.

Concentrations of Plip-P in different depth intervals are shown in Fig. 4. The distribution was very constant with time and characterized by a sharp gradient between soil and manure phase. In the soil phase a very high level of Plip-P was observed in 0–2 mm distance from the interface by Day 1, which probably originated from manure spreading out into the soil during the assembly of samples. It is not known at present to what extent Plip-P in the manure consists of undecomposed cell remains; acridine orange direct counts of bacteria were $> 10^{10}$ cells g^{-1} manure. This high level of Plip-P in the soil gradually disappeared over the following weeks, but a stimulation compared to the rest of the soil phase was still evident after 21 days. The Plip-P concentration of soil at ≥ 4 mm distance from the interface was largely unaffected by the presence of manure.

In the manure phase the slightly elevated (not significant) concentrations of Plip-P in the 0–2 mm layer compared to the rest of the manure phase sug-

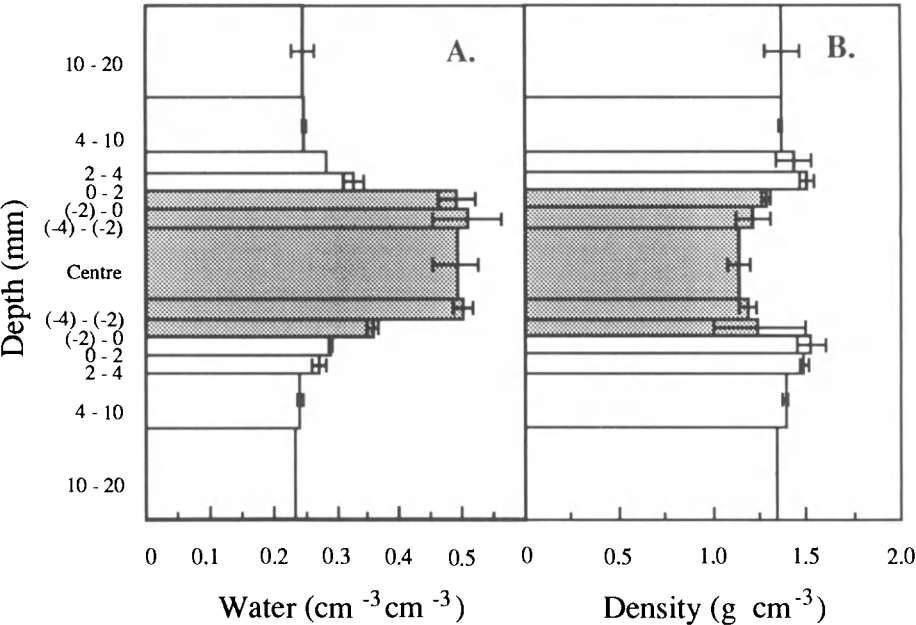


Fig. 3. Distribution of (A) water and (B) dry matter after 21 days ($n=2$). Shaded bars represent the manure phase.

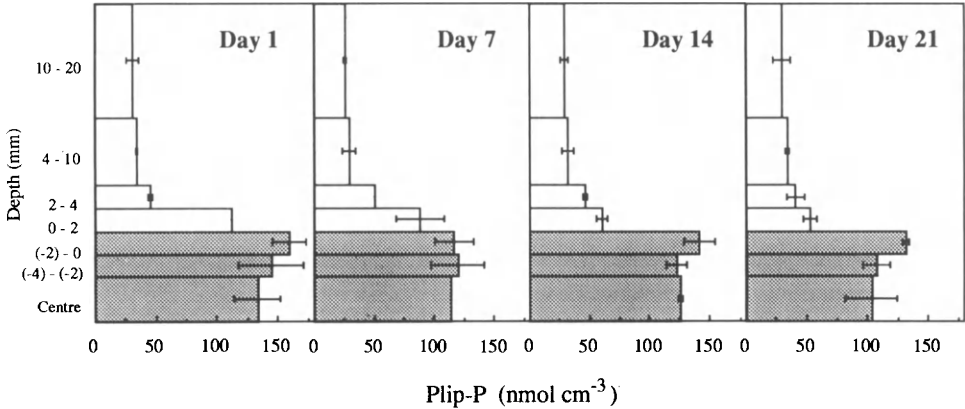


Fig. 4. Distribution of phospholipid-P in the two-phase model system during the three week period ($n=2$). Results are shown as half profiles (see text). Shaded bars represent the manure phase.

gested a stimulation of biomass close to the interface. The stimulation was less pronounced than observed previously with a different experimental design (Petersen et al., 1991a).

Figure 5A shows an oxygen profile measured after 7 days in the presence of ca. 5 mm soil phase. No decrease in oxygen concentration took place until the

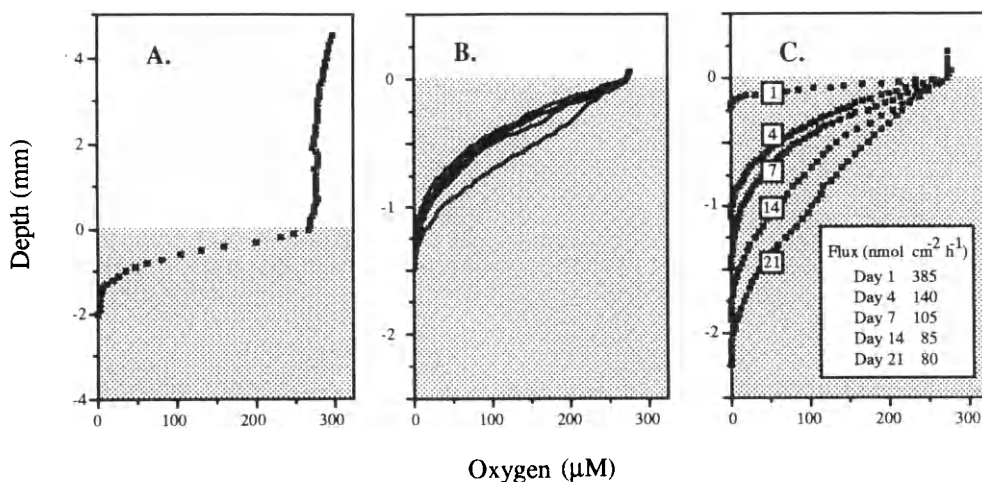


Fig. 5. Oxygen profiles measured with a microelectrode. (A) Profile measured after 7 days in the presence of 5 mm soil phase. (B) Five independent profiles measured after 7 days. (C) Representative profile from each sampling date. Inset shows the calculated diffusive flux of oxygen across the interface on each date.

interface was reached (air saturation corresponded to $275 \mu\text{M}$ oxygen). There was a good reproducibility of oxygen profile measurements, as shown in Fig. 5B, where all intact profiles of Day 7 are shown. However, the surface of the manure phase became increasingly irregular during the experiment, probably because of drying-out and/or biological activity. Large numbers of nematodes were observed at the surface after 7–14 d incubation. Intact profiles representative for the individual sampling dates were selected for calculations of diffusive flux of oxygen across the interface (Fig. 5C). The flux of oxygen (see inset) decreased from 385 to 80 $\text{nmol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ between Day 1 and Day 21. These flux rates may be compared with fluxes calculated from oxygen microprofiles in other gradient systems. Kuenen et al. (1986) measured a flux of $780 \text{ nmol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ into a nutrient-amended biofilm from a trickling filter, while Rasmussen and Jørgensen (1992), in a study of seasonal variations of oxygen uptake in a coastal sediment, found a maximum rate of ca. $50 \text{ nmol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ following the sedimentation of an algal bloom. But it should be recognized that in the soil–manure system redistribution of water may have greatly increased the total flux of oxygen into the manure-saturated zone over what was calculated from the oxygen profiles, by extending air-filled pores into the manure phase.

The maximum oxygen penetration depth was 0.15 mm by Day 1, and ca. 1 mm by Day 4. Between Day 4 and Day 21 the penetration depth only increased from 1 to ca. 2.3 mm, i.e. an anaerobic volume corresponding to 60–70% of the manure-saturated zone remained after three weeks incubation.

The fact that the soil phase was fully saturated with oxygen, while oxygen never reached deeper than 2–2.5 mm into the manure phase, suggests that aerobic decomposition of carbon substrates in the manure was largely restricted to the (–2)–0 mm depth interval during the three weeks. This is in accordance with the distribution of phospholipids, which appear to be correlated with biomass-C, as determined by CFDE; the Plip-P profiles indicated that there was a sharp gradient in biomass between this layer and the soil phase. The temporal stability of the anaerobic volume within the manure-saturated zone is without doubt important for the amount of N lost through denitrification in the field.

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Modelling diffusion and microbial uptake of ^{13}C -glucose in soil aggregates

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ABSTRACT

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A deterministic model was developed to simulate the microbial utilization and diffusion of finite amounts of substrate from the surface of a model aggregate into the intra-aggregate pore spaces. Experiments were performed for comparison with the simulation results and to estimate the effective intra-aggregate diffusion coefficient for glucose. Glucose was applied to the surfaces of sterilized and nonsterilized aggregates and the induced $^{13}\text{CO}_2$ -respiration and radial glucose distribution were measured. ATP measurements show a developing radial gradient of microbial activity decreasing from the outer to the inner part of the aggregate.

INTRODUCTION

To understand the influence of soil structure on microbial transformation or mineralization of organic compounds in heterogenous soils, it is useful to study and monitor the soil physical, chemical and microbiological processes in isolation. Using mathematical models it is possible to integrate the separately determined processes and parameters and to test concepts describing the interrelations and the mutual dependencies of the processes considered.

Heterogenous soils are often described as made up of differently sized and shaped aggregates (Rao et al., 1982; Nielsen et al., 1986), the natural habitats of soil microorganisms (Stotzky, 1972; Hattori and Hattori, 1976). Describing microbial metabolization of organics and the influence of the arrangement of microorganisms and substrates in soil aggregates is a first step towards a description of the interrelationship of soil structure and microbial processes in these soils.

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A model of a single soil aggregate was developed expressing a direct relationship between aggregate size, water content, transient intra-aggregate diffusion, concentration of metabolizable organic compounds (substrate) and microbial kinetic parameters (Priesack, 1991; Nietfeld et al., 1992). Mainly to study the denitrification process similar models were given by Leffelaar (1988), Leffelaar and Wessel (1988) and Arah (1990). Comparing simulation results of the model with experimental data from a glucose diffusion experiment performed with sterilized and nonsterilized aggregates it is the purpose, to illustrate, how microbial processes might be influenced by microscopic mass transfer, i.e. by the diffusion of the substances they transform. To explicitly account for glucose diffusion and microbial respiration kinetics, based on known parameters, the study was conducted with synthesized uniform spherical soil aggregates. These model aggregates therefore represent only a rough approximation of the average real soil aggregate which mostly has a different form and a nonuniform intra-aggregate distribution of physical, chemical and microbial parameters.

MATERIAL AND METHODS

Soil

The soil was collected from the plough layer (0–25 cm depth) of a colluvial loess loam (Tertiär – Hügelland) of a field under conventional tillage in the region of Munich near Scheyern (Germany). The C-content was 1.2%, N-content was 0.13%, the pH was 6.8 and the water content in the field was 21%. The soil material was air dried, sieved (< 2 mm) and stored at room temperature (21°C).

Aggregate preparation

Samples of 4.0 g dry weight of the sieved soil mixed with 1.5 ml distilled water were moulded into spherical soil aggregates of 1.8 cm diameter and air dried. Some of the aggregates were sterilized by exposing them to gamma-radiation (25 kGy). Sterilization was confirmed by incubation on agar dishes (Kisser-Priesack and Bode, 1991). Before using the sterilized and nonsterilized aggregates in the diffusion experiment, they were water saturated by putting them on autoclaved quartz sand with sterilized, distilled water in glass beakers covered by a LDPE foil (26–30 mm) to avoid water loss and to allow gas exchange.

^{13}C -glucose diffusion experiment

Each of the water saturated aggregates was immersed for 1–2 seconds in a 0.4% ^{13}C -glucose solution (D-glucose 1- ^{13}C , 99%, Promochem), weighed and placed in a 1.5 l air tight sterilized vial containing tissues wetted with sterilized, distilled water and a CO_2 -absorber (Natronkalk, 20 mg, Merck), to bind the CO_2 evolving from the aggregate. Incubation temperature was 21 °C. At various times after immersion, the CO_2 -absorber and the aggregate were taken out of their vials. The aggregate was weighed and frozen in liquid nitrogen. Three shells of 1–2 mm thickness were carefully peeled from the aggregate using a scalpel. Every time a shell was peeled the remaining aggregate was weighed. Then the $^{13}\text{C}/^{12}\text{C}$ -isotope ratio and the C-content of the CO_2 -absorber and of 2 aliquots of each of the 3 shells and the nucleus were measured by a mass spectrometer (Finnigan MAT, Delta E gas isotope mass spectrometer) linked to an Elemental Analyzer (Carlo Erba, ANA 1500). To get a time series this procedure was performed at 1, 2, 3, 4, 5, 6 and 24 h after immersion for the sterilized and nonsterilized aggregates and in addition for the nonsterilized aggregates at 7, 9, 12, 15, 18, 21 h. For each experiment 2–3 replicates were taken.

Microbial activity

To determine the adenosine nucleotide ATP by the method of Bai et al. (1989), water saturated aggregates were immersed as previously described in a 0.4% glucose solution (Merck) and peeled immediately and after 24 h. The soil material of three aggregates—of each aggregate two shells, the outer shell of 1 mm, the inner shell of 2 mm thickness, and the nucleus were mixed. Using two replicates for every layer the ATP of two aliquots were extracted and measured by HPLC (Bai et al., 1989; Zelles et al., 1991). The microbial biomass-C for the sieved soil was estimated by measuring the heat production after glucose amendment according to the method of Sparling (1983).

Model equations and numerical solution

The mathematical model simulates the glucose diffusion and the population dynamics of microbial biomass in a soil aggregate surrounded by a solution film. The system is described by a composite sphere, the outer being the solution film and the inner sphere representing the soil aggregate. Within the whole system, the glucose is assumed to move only by diffusion. The microorganisms are assumed to live in the soil aggregate but to be unable to enter the solution film. Inside the aggregates the microorganisms can take up glucose which they need for growth and maintenance requirements only from the soil solution. It is further assumed that the initially present microbial bio-

mass is maintained indefinitely at a constant population level by an indigenous carbon supply from the soil.

The differential equations describing the rate of change of glucose-C concentration in the composite spherical system representing a solution film of thickness d (cm) and a soil aggregate of radius a (cm) (Priesack, 1989; Priesack, 1991) are written as:

$$\frac{\partial C_0}{\partial t} = D_0 \left(\frac{\partial^2 C_0}{\partial r^2} + \frac{2}{r} \frac{\partial C_0}{\partial r} \right) \quad (1)$$

for the soil solution film ($a < r \leq a + d$), and as:

$$\theta \frac{\partial C_1}{\partial t} = D \theta \left(\frac{\partial^2 C_1}{\partial r^2} + \frac{2}{r} \frac{\partial C_1}{\partial r} \right) - \frac{\rho}{Y} \frac{\partial M}{\partial t} - \sigma \rho (M - M_A) \quad (2)$$

inside the aggregate ($0 \leq r \leq a$).

Equation (1) is a radial diffusion equation for the glucose-C concentration C_0 (mg cm⁻³) inside the solution film, where D_0 is the diffusion coefficient of glucose in free solution (cm² s⁻¹) and r is the radial distance in the solution film (cm). In eq. (2) radial diffusion is expressed by the first term of the right-hand side, where C_1 is the glucose-C concentration (mg cm⁻³) in the soil solution of the aggregate, D is the effective diffusion coefficient (cm² s⁻¹) for a nonabsorbing substance given by $D = D_0 f$ with impedance factor f (Nye and Tinker, 1977), θ is the volumetric water content (cm³ cm⁻³) and r is the radial distance in the aggregate (cm). The last two terms of eq. (2), the growth and the maintenance term, give the glucose-C utilization rate by the organisms, where M is the microbial biomass-C in the aggregate per dry weight of soil (mg g⁻¹) and ρ is the soil dry weight per unit volume of soil (g cm⁻³). Y is the yield factor (mg mg⁻¹) giving the amount of microbial biomass-C produced per glucose-C used for growth and σ is the maintenance coefficient (mg mg⁻¹ s⁻¹) determining the rate of substrate utilization for the maintenance metabolism.

Assuming the growth dynamics follow Monod kinetics modified by the requirement for maintenance energy the equation for microbial growth is given by the rate of change of microbial biomass:

$$\partial M / \partial t = \mu M \quad (3)$$

and the specific growth rate:

$$\mu = \mu_{\max} C_1 / (C_1 + K) - \omega \quad (4)$$

where $\mu_{\max}$ denotes the maximal specific growth rate (s⁻¹), K is the affinity constant per unit volume (mg cm⁻³) written as $K_C \rho / \theta$ with affinity constant K_C per unit dry weight of soil (mg g⁻¹) and ω is the microbial decay coefficient (s⁻¹) given by $\omega = \sigma Y (1 - M_A / M)$ maintaining the initial microbial bio-

mass-C concentration (Darrah, 1991b). The initial conditions are given by the constant glucose-C concentration C_1 (mg cm^{-3}) in the solution film:

$$C_0 = C_1, \quad a < r \leq a + d \quad (5)$$

by the condition of no glucose-C being initially present in the aggregate:

$$C_1 = 0, \quad 0 \leq r \leq a \quad (6)$$

and by the initial constant biomass-C concentration M_A per dry weight of soil (mg g^{-1}):

$$M = M_A, \quad 0 \leq r \leq a \quad (7)$$

At the outer surface of the water film it is assumed that no glucose enters or leaves the system of the composite sphere giving the first boundary condition:

$$\partial C_0 / \partial r = 0, \quad r = b \quad (8)$$

The second and third boundary condition state the equality of the glucose solute concentrations and the continuity of the diffusive flux at the interface between solution film and soil aggregate:

$$C_0 = C_1, \quad r = a \quad (9)$$

$$D_0 \frac{\partial C_0}{\partial r} = D\theta \frac{\partial C_1}{\partial r}, \quad r = a \quad (10)$$

The last boundary condition is similar to the first describing no glucose can enter or leave through the centre of the aggregate:

$$\partial C_1 / \partial r = 0, \quad r = 0 \quad (11)$$

To solve this initial boundary value problem for $C(r, t)$ and $M(r, t)$, the equations were discretized following a fully implicit finite difference scheme (Richtmeyer and Morton, 1967) and the resulting nonlinear tridiagonal equation system is solved by Newton–Raphson iteration. The boundary conditions at the interface of the solution film and the aggregate were dealt with according to Crank (1956).

Estimation of the effective diffusion coefficient

The solution of the above equations requires an estimate of the impedance factor f . To estimate this parameter the analytical solution for the case of no microbial degradation and no adsorption (Priesack, 1991) was fitted to the experimental data of glucose diffusion in the sterilized aggregates. For this purpose the impedance factor f was adjusted so as to obtain the best fit of the simulated to the measured concentrations. Depending on the parameter f , the simulated relative glucose concentrations for the 3 shells and the nucleus were

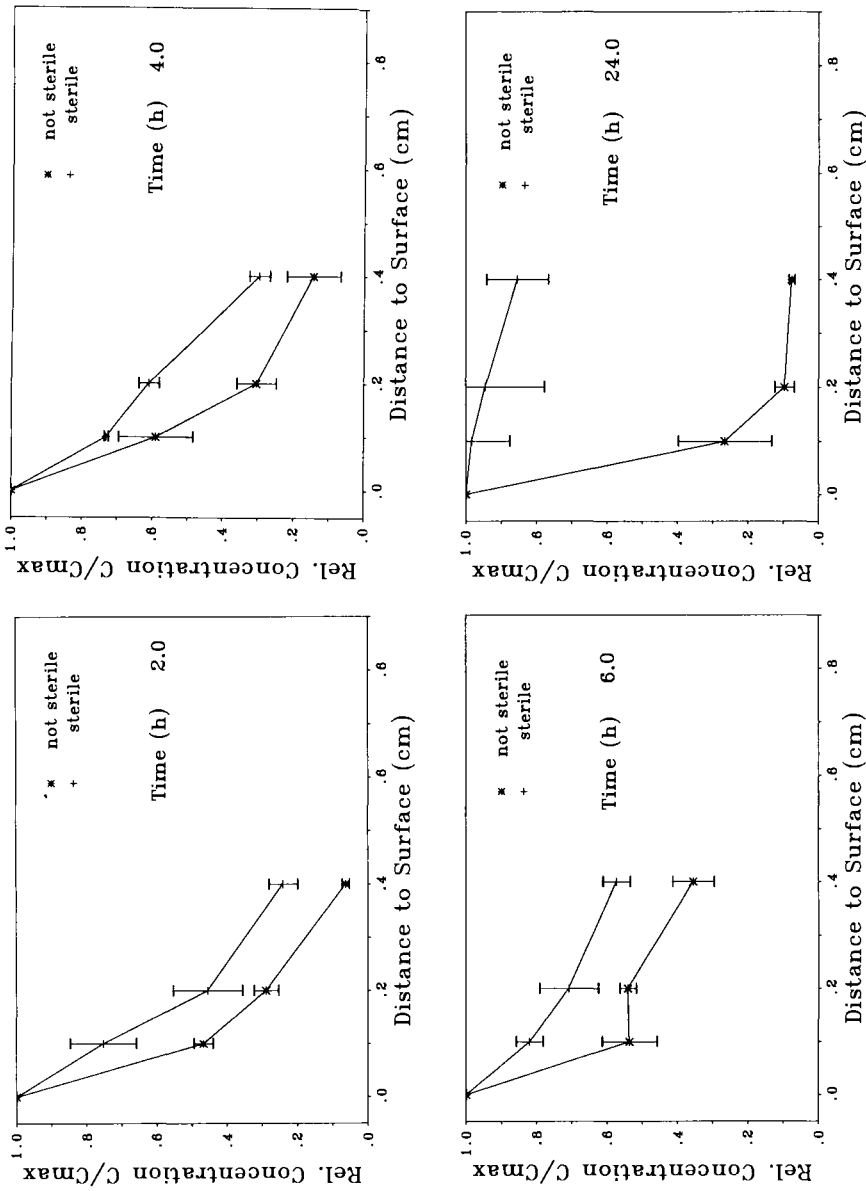


Fig. 1. Relative ^{13}C -glucose concentrations within sterilized and nonsterilized spherical soil aggregates of 1.8 cm diameter versus radial distance to the aggregate surface for 2, 4, 6 and 24 h after immersion in 0.4% ^{13}C -glucose solution.

calculated by evaluating the corresponding 4 integrals of the analytical solution. The resulting values were fitted to the measured relative ^{13}C -glucose concentrations inside the sterilized aggregates using a least square method. Since for each time values of 3 replicates were measured, 3^6 different time series could be composed. By gathering time series which have no samples in common, so that not two of the collected series have values of the same aggregate, we got 3^5 triples of independent time series. For each triple a mean value of the results of fitting runs to the three time series was calculated. Finally the median of the 3^5 mean values was taken as estimated impedance factor.

Statistics

If not otherwise stated all measured values are given as mean values followed by the standard error and the number of replicates. Bars in all figures represent standard errors.

RESULTS AND DISCUSSION

The simulations given by Priesack (1991) and Nietfeld et al. (1992) show substrate exhaustion by microorganisms close to the surface for larger soil aggregates (of radius > 0.5 cm). Thus an aggregate center may not be reached by substrate diffusion from the aggregate surface. To investigate this phenomenon, Figs. 1–3 give a data set on glucose distributions within sterilized and nonsterilized soil aggregates at different times between 1 and 24 hours after immersion in 0.4% ^{13}C -glucose solution. The spherical aggregates had a radius of 0.9 cm and a soil dry weight of 4 g. The mean value of the amount of ^{13}C -glucose taken up by the aggregate during immersion was 389 ± 15 μg , $n=68$. For a 0.4% glucose concentration of the solution film this corresponds to a mean solution film thickness $d=0.04$ cm and an initial solution film concentration of $C_i=0.4$ mg cm^{-3} , used as input parameter values for the simulations. This solution film thickness was assumed to account for the water film volume from which the glucose amount was absorbed. The average volumetric water content θ was 0.393 ± 0.002 , $n=79$. For the radial relative ^{13}C -glucose distribution C/C_{max} , C_{max} being the total concentration in the outer aggregate shell, Fig. 1 shows significant differences between sterilized and nonsterilized aggregates at all times, most obviously after 24 hours. Using the values of these relative concentrations for the sterilized aggregates, the impedance factor f was estimated by the procedure reported above to be 0.283 ± 0.018 , $n=3$. This value is comparable to values of impedance factors for cylindrical aggregates calculated by Darrah (1991a) for a lower volumetric water content and a higher bulk density of a sandy loam. The resulting diffusion curves of the relative glucose concentration C/C_{tot} within the sterilized aggregates were calculated with the estimated impedance factor and

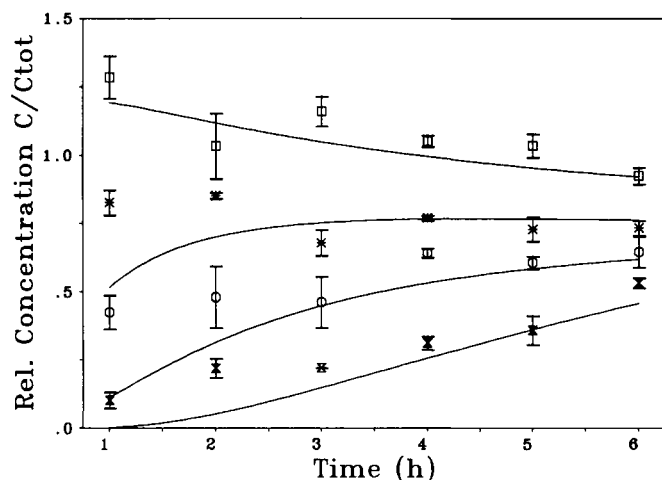


Fig. 2. Relative ^{13}C -glucose concentrations within sterilized spherical soil aggregates of 1.8 cm diameter versus time after immersion in 0.4% ^{13}C -glucose solution: measured data [at 0.0–0.1 cm ($\square$), 0.1–0.2 cm ($+$), 0.2–0.4 cm ($\circ$), 0.4–0.9 cm ($\times$) distance to the surface] and fitted analytical solution (—).

plotted versus time (Fig. 2), where C_{tot} represents the concentration for the whole aggregate. The measured values for the time points 1 h and 2 h are considerably higher than the calculated values. This might be explained by solute flux into the first shell during the procedure of immersion. As a consequence, the impedance factor could have been overestimated representing not only mass transfer by diffusion but also by flux.

In comparison to Fig. 2, Fig. 3 shows the means of the relative concentrations C/C_{tot} within the nonsterilized aggregates. Here C_{tot} is given by the glucose amount taken up by the aggregate after immersion related to the aggregate volume. For the measured values, C_{tot} was calculated from the amount of glucose inside the aggregate and the amount of glucose corresponding to the ^{13}C amount taken up by the CO_2 -absorber.

Table 1 summarizes the parameter values used for the simulation. The initial microbial biomass-C per dry weight of soil within the aggregate was assumed to be constant throughout the aggregate and equal to the measured biomass-C concentration $260.7 \pm 2.4 \mu\text{g g}^{-1}$, $n=4$ of the sieved soil. The values obtained by solving eqs. (1–4) using the numerical solution represent glucose concentrations in the soil solution of the aggregate, whereas the measured data are the glucose concentrations in the soil derived from the total ^{13}C -concentrations. After about 10 to 12 hours, most of the glucose of the outer aggregate shell has been taken up and immobilized by the microorganisms, since transport into the next inner shell (0.8–0.7 cm) has apparently stopped. Respiration has also stopped, possibly due to a lack of substances

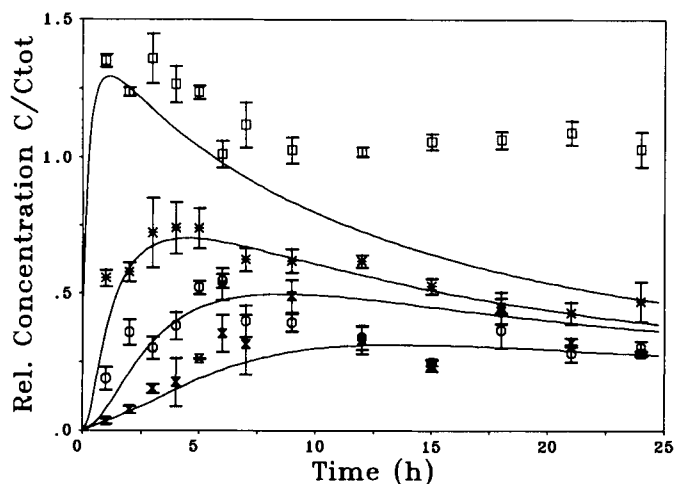


Fig. 3. Relative ^{13}C -glucose concentration within nonsterilized, spherical soil aggregates of 1.8 cm diameter versus time after immersion in 0.4% ^{13}C -glucose solution: measured [at 0.0–0.1 cm ($\square$), 0.1–0.2 cm ($+$), 0.2–0.4 cm ($\circ$), 0.4–0.9 cm ($\times$) distance to the surface] and simulated (—).

like N, P, K or oxygen limiting the growth and metabolic processes of the microorganisms. As the model describes only the total microbial glucose-C uptake from the soil solution, it does not distinguish between respiration and immobilization of the glucose derived carbon. Hence the simulated results give only values for the mobile, diffusing glucose-C concentrations in the soil solution. In contrast, the measured results show the respired glucose-C which left the aggregate as CO_2 and the carbon from the added glucose remaining in the aggregate. They do not distinguish between immobilized carbon and mobile glucose-C in the soil solution. This could explain the discrepancy between the simulated curve and the data for the first shell (0.9–0.8 cm) of the aggregates if the difference represents the amount of immobilized glucose-C. For the next shells and the nucleus of the aggregate the calculated solute concen-

TABLE 1

Input parameter values for simulations

$a = 0.9 \text{ cm}$	$\theta = 0.39$	$\mu_{\max} = 0.02 \text{ h}^{-1} \text{ }^b$
$d = 0.04 \text{ cm}$	$\rho = 1.31 \text{ g cm}^{-3}$	$\sigma = 0.01 \text{ h}^{-1} \text{ }^c$
$D_0 = 6.73 \cdot 10^{-6} \text{ cm}^2 \text{ s}^{-1} \text{ }^a$	$C_1 = 0.4 \text{ mg cm}^{-3}$	$Y = 0.5 \text{ }^b$
$f = 0.28$	$M_A = 0.261 \text{ mg g}^{-1}$	$K_C = 0.06 \text{ mg g}^{-1} \text{ }^b$

References:

^aWeast (1964).

^bAnderson and Domsch (1986).

^cAnderson and Domsch (1985).

tration curves fit quite well to the measured concentrations. During the first 5 hours the concentration distribution is mainly determined by the diffusion process. After 5 hours, the glucose concentration of the inner layers decreases and no immobilization can be seen corresponding to the simulation. Also for the outer shell, the microbial glucose uptake seems to be correctly simulated, since the calculation of the diffusion process into the next inner shell leads to correct glucose concentrations for the inner aggregate. As a result of this glucose uptake close to the aggregate surface the glucose concentration in the aggregate centre remains low compared to that in the sterilized aggregates, where after 24h the constant equally distributed steady state concentration is almost reached.

Simulated results given in Table 2 illustrate the influence of the most relevant factors on the penetration of glucose and the microbial growth response in the aggregate. The relative glucose-C penetration depth d_{cp}/a (cm cm⁻¹) and the relative growth response depth d_{gr}/a (cm cm⁻¹) were calculated for different values of the aggregate radius a , the impedance factor $f=D/D_0$, the initial film solution concentration C_1 and the maximal specific growth rate μ_{max} . The glucose-C penetration depth d_{cp} (cm) is defined as the maximal distance from the aggregate surface, where a concentration of the penetrating glucose-C higher than 1% of the initial film solution concentration C_1 is reached. In a similar way, the relative growth response depth d_{gr} (cm) is given by the maximal distance from the aggregate surface where the initially present

TABLE 2
Relative glucose-C penetration depth and relative growth response depth

$d/a=0.05^*$	C_1	$\mu_{\max}=0.2\text{ h}^{-1}$		$\mu_{\max}=0.02\text{ h}^{-1}$	
	(mg cm^{-3})	d_{cp}^*	d_{gr}^*	d_{cp}^*	d_{gr}^*
$D/D_0=0.3^*$					
$a=1.0\text{ cm}$	0.5	0.33	0.48	1.00	1.00
	0.1	0.29	0.28	1.00	1.00
$a=0.5\text{ cm}$	0.5	0.68	1.00	1.00	1.00
	0.1	0.58	0.53	1.00	1.00
$D/D_0=0.03^*$					
$a=0.5\text{ cm}$	0.5	0.22	0.31	0.70	1.00
	0.1	0.19	0.19	0.60	0.45
$a=0.25\text{ cm}$	0.5	0.41	0.62	1.00	1.00
	0.1	0.36	0.34	1.00	1.00
$\rho=1.35\text{ g cm}^{-3}$		$M_A=0.50\text{ mg cm}^{-3}$		$Y=0.5^*$	
$\theta=0.25^*$		$K_C=0.05\text{ mg cm}^{-3}$		σ	
				$=0.01\text{ h}^{-1}$	

*Dimensionless parameters.

microbial biomass-C has grown more than 1%. The simulated results indicate that glucose can diffuse to centers of bigger aggregates ($a \geq 0.5$) only if the diffusion is fast enough ($f=0.3$) and microbial growth is slow ($\mu_{\max}=0.02 \text{ h}^{-1}$). For slow diffusion ($f=0.03$) even for smaller aggregates there may exist an inner region without glucose and without growth if the substrate input is low ($C_1=0.1 \text{ mg cm}^{-3}$) and the glucose uptake by the microorganisms is high ($\mu_{\max}=0.2 \text{ h}^{-1}$). Especially when the substrate is not only taken up by microorganisms but also adsorbed to the soil matrix there might be inner regions without induced growth also in aggregates of diameter smaller than 0.1 cm (Priesack, 1989).

In Fig. 4 the respired ^{13}C -glucose, given as a percentage of the total ^{13}C -glucose taken up by the aggregate, is plotted versus time. With a correlation of $r=0.973$ a linear regression estimates a ^{13}C -glucose respiration rate of 0.72% per hour for the first day after glucose addition. This corresponds to the $^{14}\text{CO}_2$ release reported by Van Veen et al. (1985) for a sandy loam and a clay soil after addition of ^{14}C -glucose.

The ATP concentrations (Fig. 5) show a developing radial gradient of microbial activity decreasing from the outer to the inner part of the aggregate. The initial slight gradient might have been developed during the saturation of the aggregate where the microorganisms at the aggregate surface possibly had a better oxygen supply. Twenty-four hours after glucose amendment the ATP concentrations are higher than at the beginning of the diffusion experiment. This indicates the formation of an outer aggregate region of higher microbial activity than in the rest of the aggregate.

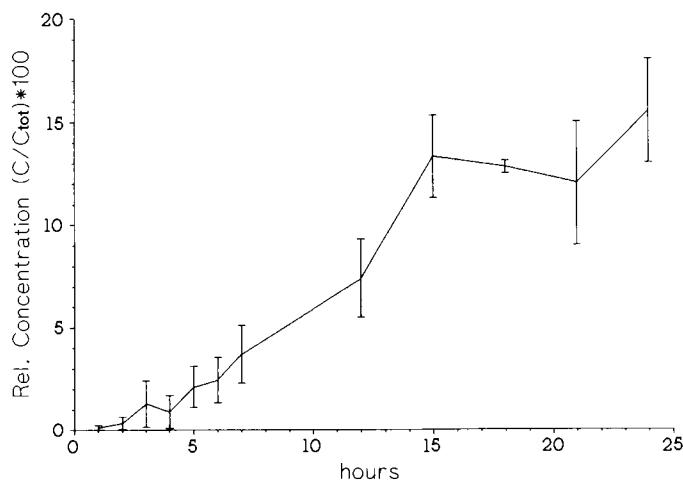


Fig. 4. Respired ^{13}C -glucose as percentage of ^{13}C -glucose taken up during immersion.

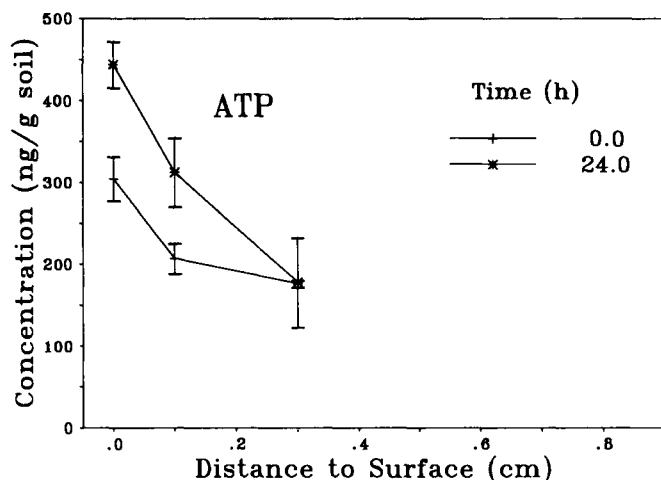


Fig. 5. Radial ATP distribution within nonsterilized, spherical soil aggregates of 1.8 cm diameter after 0.0 h and 24.0 h of glucose diffusion.

CONCLUSIONS

The model developed to describe substrate diffusion and substrate uptake by microorganisms in soil aggregates was found to represent glucose diffusion and microbial glucose uptake correctly using data from the literature to simulate the kinetics of glucose uptake. For a better comparison with the measured ^{13}C concentration data it is necessary to include a description of the processes of immobilization and CO_2 -respiration into the model.

The results show that the dynamics of substrate transport and microbial transformation within the aggregate can influence the distribution of microbial activity. The substrate supply at the aggregate surface induced higher activity in the outer parts than in the inner parts of the aggregate. This may result in the development of substrate-starved zones at the centres of larger aggregates. In heterogeneous soils consisting of long lived aggregates, the surface regions of the aggregates are probably the regions of higher microbial biomasses and activities.

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Analysis of the microbial nutrient status in soil microcompartments: earthworm faeces from a basalt–limestone gradient

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ABSTRACT

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The nutrient status of the microbial community in casts of earthworms (*Octolasion lacteum* (Örley)) and in soil from three different beechwood (*Fagus sylvatica* L.) sites of a basalt–limestone gradient (basalt, intermediate and limestone site) was investigated by analysing the respiratory response of the microflora to glucose (C) and nutrient (N and P) amendments over a period of about 40 h. Microbial respiration was measured as O₂ consumption by automated microcompensation. Cumulative additional microbial respiration up to the maximum respiration rate was used as a measure of microbial growth ability in C, CN, CP and CNP amended samples.

Organic carbon in soil of the limestone site was twice that of the basalt and intermediate site and the initial respiratory response of soil samples from the limestone site exceeded that of samples from the basalt and intermediate site by a factor of about 2. In soils of the basalt and intermediate sites C availability limited microbial growth, whereas in soil of the limestone site microbial growth was limited by the availability of P. In C amended soils of the basalt and intermediate sites microbial growth was enhanced strongly by additional N.

The gut passage of the soil reduced microbial N deficiency in soil from the basalt and intermediate sites and reduced P deficiency in limestone soil. In general, microbial growth in earthworm casts was limited by C availability.

Microbial biomass was similar in soil and earthworm casts. Soil from the limestone site contained twice the biomass of soils from the basalt and intermediate sites. However, the ratio of C_{mic}/C_{org} was similar in soil from each of the study sites. A ratio of C_{mic}/C_{org} close to 0.015 is assumed to be typical for mull beechwood soils.

INTRODUCTION

Ecosystem processes can be analysed at different scales (O'Neill et al., 1986). The aim of many macro-scale analyses of ecosystem processes is the

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determination of carbon, nutrient and proton budgets. Internal turnover processes are treated as a black box. The macro-scale approach has been particularly successful at describing the state of ecosystems and predicting alterations due to disturbances (Ulrich, 1987). However, for a closer understanding of the mechanisms affecting macro-scale budgets, it is necessary to analyse internal turnover processes.

An overwhelming number of soil organisms is involved in carbon, nutrient and proton turnover, and all of their interactions cannot be studied in detail (Schaefer, 1990). Micro-scale analysis of ecosystems must therefore focus on specific internal processes, which can be linked to macro-scale phenomena.

In the present study the effect of earthworms on the microbial nutrient status in soil of a beechwood growing on a basalt–limestone gradient is analysed. In terms of biomass the saprophagous community at the study sites is dominated by earthworms (Schaefer, in prep.) and earthworms are assumed to be important soil forming agents. The analysis of the effects of earthworms on microbial nutrient status may therefore lead to a better understanding of the development of the present nutrient status of the microbial community at the study sites. In addition, earthworms are considered to be model organisms and the analysis of their impact on soil microorganisms may reflect the general possibilities of saprophagous soil invertebrates to affect the microbial nutrient balance.

The analysis of the impact of soil invertebrates on microbial nutrient status is considered to be particularly useful because the nutrient status of the soil microflora provides a functional parameter of high order. Results of analysis on a microsite level may therefore be linked to processes observed on the macro-scale (Anderson, 1988).

MATERIALS AND METHODS

The substrates

Soil samples were taken from three different beechwood sites located on a basalt hill ("Kleiner Gudenberg") near Kassel (Germany). The sites form a gradient from basalt (upper part of the hill) to limestone (lower part of the hill) (more details of the sites are given by Eichhorn, 1991). Soils from the upper part (basalt site), from a site between upper and lower part (intermediate site) and from the lower part of the hill (limestone site) were investigated. Soil samples from the upper 3 cm of the mineral soil (Ah horizon) were taken in June 1991 and passed through a 4 mm sieve. The water contents of the samples from the basalt, intermediate and limestone sites were 56%, 58% and 70% of dry weight respectively. Organic carbon contents of the soil samples from the basalt and intermediate site were similar and about 0.5 that

TABLE 1

Organic carbon and nitrogen content, C-to-N ratio and pH (H₂O measurement) of soil samples taken from three different sites of a basalt-limestone gradient (means of three replicates)

Site	Carbon content (%)	Nitrogen content (%)	C-to-N ratio	pH
Basalt	7.1	0.51	13.9	5.5
Intermediate	5.9	0.39	15.2	5.3
Limestone	12.7	0.83	15.3	7.6

of the limestone site (Table 1). The parent rock of the three sites is reflected by the pH of the soil samples (Table 1).

In order to obtain earthworm casts, cages were filled with soil of the three sites (3 replicates each) in which the borrowing activity of earthworms could be observed (cf. Scheu, 1987a). Ten specimens of the endogenic earthworm species *Octolasion lacteum* (Örley) were introduced per cage. The cages were incubated at 15°C in the laboratory in permanent darkness for 8 days. Earthworm casts deposited during the incubation period (i.e. average age of 4 days) were sampled and used as substrates. Control soil samples were taken from cages treated in the same way but without earthworms. Both substrates were stored at least for 2 and maximally for 10 days at 22°C prior to the analysis. Pre-incubation of at least 2 days was done to adapt the soil materials to temperature conditions of the respiratory measurement. In some cases it was necessary to store the material some days longer because of limitations of the respirometer.

Analysis of the microbial nutrient status

The microbial nutrient status was determined measuring the respiratory response of the soil microflora to glucose and nutrient (N and P) amendments for about 40 h. During the first 4 to 6 h glucose (and nutrient) amendment results only in an increase in the metabolism of the existing soil microorganisms (maximal initial respiratory response, MIRR; Anderson and Domsch, 1978), the MIRR thus reflects the microbial biomass. After this initial phase, the microorganisms begin to grow and glucose oxidation increases again. The ability of the soil microflora to grow depends not only on the availability of carbon (glucose) but also on that of nutrients. Hence, the extent of glucose oxidation during growth reflects the amount of nutrients available to the microflora. The cumulative additional microbial respiration (following the initial response) up to the maximum respiration rate (AMR) was therefore used to characterize the microbial nutrient status.

Eight thousand ppm of glucose was added to the substrates. Nitrogen (as

NH_4NO_3) and P (as Na_2HPO_4) were added respecting a C:N:P ratio of 10:2:1, which is in the range of microbial tissue (Anderson and Domsch, 1980). Glucose and nutrients were added as aqueous solutions to increase the water content of the soil samples to 100% of dry weight.

The effect of the following amendments on the respiratory response in soil and earthworm casts from the basalt, intermediate and limestone site was analysed (three replicates each):

C: glucose addition only;

CN: addition of glucose and nitrogen;

CP: addition of glucose and phosphorus;

CNP: addition of glucose, nitrogen and phosphorus.

Data on AMR were analysed by four way analysis of variance. Factors were SUBSTRATE (soil and earthworm casts), SITE (basalt, intermediate and limestone site), NITROGEN (with and without N amendment) and PHOSPHORUS (with and without P amendment).

Measurement of oxygen consumption

The respiratory response of the soil microflora to glucose (and nutrients) was measured as O_2 consumption by automated microcompensation (Scheu, 1992). Therefore, soil samples equivalent to 2 g dry weight were placed in small vessels which were attached to an electronic pressure detector. CO_2 produced in the vessel was absorbed in alkali solution. Each vessel containing soil samples was connected to an electrolytic chamber. O_2 content in the system was maintained constant by electrolytic O_2 release from a saturated CuSO_4 solution. The apparatus consisted of 16 respirometer units and was controlled by computer.

RESULTS

Maximal initial respiratory response

The MIRR following glucose amendment was similar in soil from the basalt site and the intermediate site (Table 2). In soil from the limestone site MIRR was almost twice as high. For the three sites MIRR was similar for soil and casts (Table 2), indicating that existing microbial biomass in soil is only slightly affected by the gut transit of earthworms.

Microbial respiration during growth

The AMR was strongly affected by the factors investigated. An overall 98.1% of the variation could be accounted for by the treatments (Table 3). SITE and NITROGEN had the most pronounced influence and accounted for 36% and

TABLE 2

Maximal initial respiratory response following glucose amendment in earthworm casts and soil from three sites of a basalt–limestone gradient (basalt, intermediate and limestone site) (means of three replicates with standard deviation).

	Maximal initial respiratory response ($\mu\text{g O}_2 \text{ g}^{-1} \text{ h}^{-1}$)					
	Basalt site		Intermediate site		Limestone site	
	Mean	SD	Mean	SD	Mean	SD
Soil	33.1	2.2	33.7	4.3	69.4	2.7
Casts	32.9	4.7	37.9	3.4	76.0	7.5

TABLE 3

Four-way ANOVA with square root transformed data on the additional microbial respiration caused by glucose and nutrient applications (AMR; for details see text)

Source of variation	SS	DF	F
SUBSTRATE	571	1	231***
SITE	2284	2	463***
NITROGEN	1843	1	747***
PHOSPHORUS	45	1	18***
SUBSTRATE×SITE	15	2	3 n.s.
SUBSTRATE×NITROGEN	697	1	283***
SUBSTRATE×PHOSPHORUS	48	1	19***
SITE×NITROGEN	254	2	52***
SITE×PHOSPHORUS	161	2	33***
NITROGEN×PHOSPHORUS	3	1	1 n.s.
SUBSTRATE×SITE×NITROGEN	253	2	51***
SUBSTRATE×SITE×PHOSPHORUS	48	2	8***
SUBSTRATE×NITROGEN×PHOSPHORUS	1	1	1 n.s.
SITE×NITROGEN×PHOSPHORUS	28	2	6**
SUBSTRATE×SITE×NITROGEN×PHOSPHORUS	8	2	2 n.s.
Residual	118	48	
Total	6377	71	

Factors were SUBSTRATE (soil and earthworm casts), SITE (basalt, intermediate and limestone site), NITROGEN (additional N amendment) and PHOSPHORUS (additional P amendment).
*** $P < 0.001$, ** $P < 0.01$, n.s. $P > 0.05$).

30% of the variation, respectively ($P < 0.001$). Nine percent of the variation was accounted for by SUBSTRATE ($P < 0.001$). The interaction of the factors NITROGEN×SUBSTRATE and NITROGEN×SITE accounted for an additional 11% and 4% of the variation, respectively ($P < 0.001$). For the three-way

interactions the SUBSTRATE×NITROGEN×SITE combination had the most pronounced influence and accounted for 4% of the variation ($P < 0.001$). In general, the strong effects of N fertilization reflects the great importance of N for microbial growth in the soils studied.

Glucose amendment

Glucose amendment enabled the microflora in soil of the basalt site and the intermediate site to grow, whereas the microflora in soil from the limestone site was not able to grow with glucose addition only (Fig. 1). Hence, the soil microflora of the limestone site was limited by another element than C. In earthworm casts microbial growth was generally limited by C availability. In comparing soils of the three sites, AMR values were considerably higher in

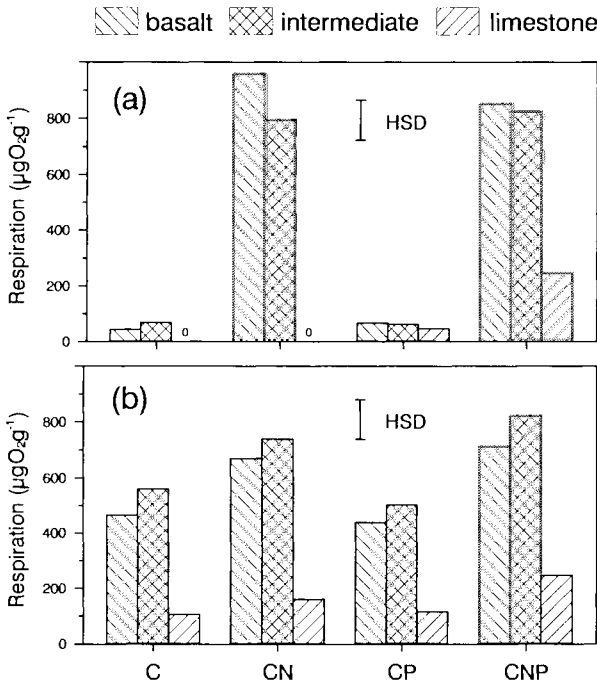


Fig. 1. Additional microbial respiration during growth (AMR; see text) caused by amendment of glucose (C), glucose and nitrogen (CN), glucose and phosphorus (CP) and glucose, nitrogen and phosphorus (CNP) in (a) soil and (b) earthworm casts from three sites of a basalt–limestone gradient (means of three replicates). HSD: Tukey's honestly significant difference.

earthworm casts, indicating additional microbial nutrient supply due to the gut transit of soil from each of the study sites.

Glucose and nitrogen amendment

In soil from the basalt site and the intermediate site, microbial growth was strongly increased by additional N amendment (22 and 12 fold increase in AMR respectively; Fig. 1). In contrast, additional N supply did not affect microbial growth in limestone soil.

As shown previously, the effect of additional N amendment depended not only on the soils studied (SITE $\times$ NITROGEN) but also on the substrates used (SUBSTRATE $\times$ NITROGEN). AMR of casts was increased by additional N amendment for the basalt, intermediate and limestone soils by factors of 1.4, 1.3 and 1.5 respectively. Therefore, microbial growth in earthworm casts supplemented with C was limited by N for the three sites of study. However, the increase in AMR due to additional N amendment to casts produced in soils from the basalt and intermediate sites was considerably lower than in bulk soils of these sites, indicating enhanced N supply in earthworm casts.

Glucose and phosphorus amendment

The effect of additional P amendment (PHOSPHORUS) depended on the factors SITE and SUBSTRATE ($P < 0.001$; Table 3). Microbial growth in soil from the basalt and intermediate site was affected by additional P amendment only slightly (Fig. 1). In contrast, in limestone soil additional P amendment had a pronounced effect and enabled the microorganisms to grow. Hence, the microflora in bulk soils of this site was limited by P availability.

The AMR of earthworm casts supplemented with glucose and P was almost identical to that of casts amended with glucose only (Fig. 1). Therefore, additional P amendment did not affect microbial growth in earthworm faeces, indicating sufficient P supply for microbial growth in casts of earthworms.

In comparison to CP fertilized soil microbial growth was more pronounced in CP fertilized casts (6.6, 8.4 and 2.5 fold increase in AMR in earthworm casts from the basalt, intermediate and limestone site in comparison to the respective bulk soils; Fig. 1). This indicates an enhanced supply of nutrients other than P in casts voided in soil of each of the study sites.

Glucose, nitrogen and phosphorus amendment

In general, microbial growth was not affected significantly by the interaction of the factors NITROGEN $\times$ PHOSPHORUS (Table 3). The significant three way interaction ($P < 0.01$) of factors SITE $\times$ NITROGEN $\times$ PHOSPHORUS reflects that microbial growth in CNP fertilized limestone soil was more pro-

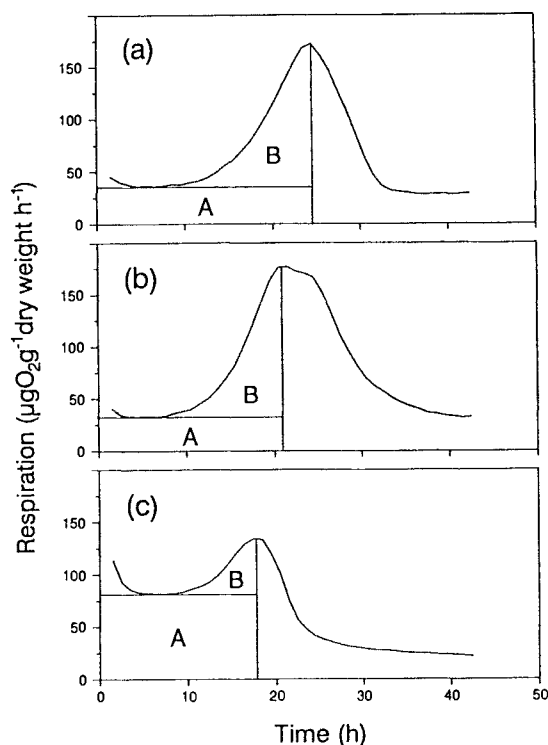


Fig. 2. Microbial respiration rates in soil from three sites of a basalt–limestone gradient following amendment of glucose, nitrogen and phosphorus. (a) Basalt site; (b) intermediate site; (c) limestone site. A: Microbial respiration due to energy metabolism (level of the maximal initial respiratory response; MIRR) B: Additional respiration due to microbial growth (AMR).

nounced than in CN fertilized soil (no microbial growth) and CP fertilized soil (AMR of 19% of CNP fertilized soil) of this site (Fig. 1). This indicates that microbial growth in CP amended limestone soil was limited by N availability.

AMR in CNP fertilized casts and soil of the basalt and intermediate site exceeded considerably that of casts and soil of the limestone site (Fig. 1). It may therefore be concluded that microbial growth due to glucose and nutrient additions was less effective in limestone soil. However, lower additional glucose oxidation during growth in limestone soil is assumed to be caused by a more rapid glucose depletion due to higher glucose utilization for energy metabolism. As already shown, microbial biomass was about twice as high in soil of the limestone site than in soil of the basalt and intermediate site, i.e. the MIRR in the limestone soil was about twice that in the soil of the basalt and intermediate site (Table 2). Therefore, lower AMR in the limestone soil resulted from higher glucose oxidation due to the energy metabolism of the two-

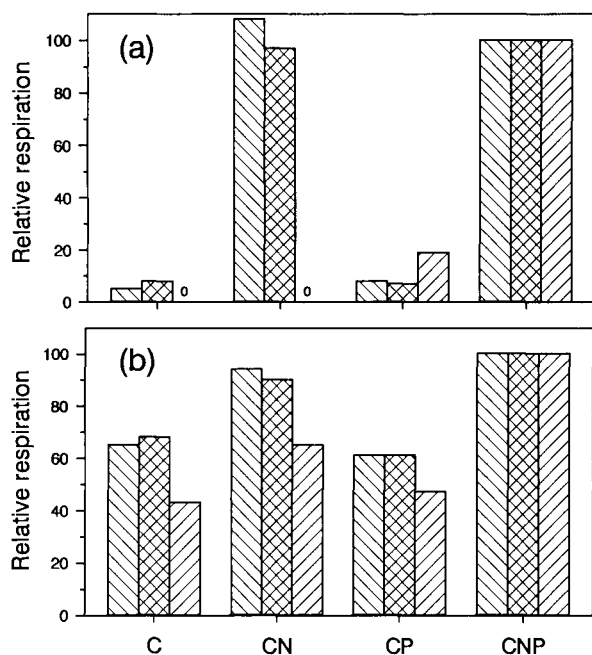


Fig. 3. Additional microbial respiration during growth (AMR; see text) caused by amendment of glucose (C), glucose and nitrogen (CN), glucose and phosphorus (CP) and glucose, nitrogen and phosphorus (CNP) in (a) soil and (b) earthworm casts from three sites of a basalt–limestone gradient as percentages of AMR of CNP amended soil and casts respectively. For legend see Fig. 1.

fold higher microbial biomass (Fig. 2). Differences in AMR values of soil samples (supplemented with the same amount of glucose) containing very different amounts of microbial biomass must be interpreted with caution. The problem may be overcome by equalling AMR of CNP fertilized samples to 100. The relative effect of C, CN and CP amendment on microbial growth can be calculated then as percentages of CNP fertilized samples (Fig. 3). This may facilitate comparisons of C and nutrient effects on microbial growth in soils with different microbial biomass.

DISCUSSION

The nutrient limitation of soil microorganisms is considered to be a general property of ecosystems. Alterations are expected to occur only slowly because the turnover of the soil microflora is low (Jenkinson and Ladd, 1981). The element limiting microbial growth and the nutrient status of the soil microflora are therefore parameters of high-order integrating micro-scale alterations in space and time (cf. Anderson, 1988). In a previous study Scheu

(1990) analysed changes in the microbial nutrient status during secondary succession in soil from different sites on limestone. It was concluded in that study, that the limiting element for microbial growth changes during secondary succession of limestone ecosystems from C in early stages to P in the beechwood climax ecosystem. The nutrient status of the soil microflora of the sites investigated in the present study supports this hypothesis. The three beechwood sites formed a gradient from basalt to limestone and the soil microflora of the limestone site was limited by P. The microflora of the basalt and intermediate site was limited by C. This indicates that the element limiting microbial growth is related to parent rock material.

Microbial growth in soil of the basalt and intermediate site was limited by C availability, but additional C amendment caused only little growth in comparison to CNP fertilization (AMR of 5% and 8% of CNP fertilized samples respectively). This indicates that the supply of at least one of the macronutrients N or P was low in soil from both sites. The comparison of soils amended with C and N or C and P indicated that the limiting element for microbial growth in C supplemented samples was N. Hence, in contrast to soil of the limestone site where P was the most important element limiting microbial growth, in soil of the basalt and intermediate site microbial growth was restricted mainly by N availability. Microbial N deficiency was particularly unexpected in soil of the intermediate site, because at this site a dense herb layer consisting mainly of *Urtica dioica* L. is present, indicating excessive N supply. The low impact of additional P amendment on microbial growth in soils from the basalt and intermediate site was also unexpected, because in these soils the level of Olsen extractable P is close to the low level observed in the soil of the limestone site (Scheu, unpubl.).

Effects of the passage of soil through the gut of earthworms on the microbial nutrient status were investigated in the present study using faeces particles deposited during 0–8 days of incubation. It was observed in a previous study (Scheu, 1987b) that the microbial nutrient demands can alter rapidly in casts of endogenic earthworms; however, changes in the nutrient limitation of soil microorganisms caused by the gut passage lasted for more than 30 days. Hence, faeces particles of a mean age of 4 days were considered to be useful materials to investigate general effects of the gut passage on the microbial nutrient status of different soils.

The gut passage of soil through earthworms eliminated microbial P limitation in limestone soil and increased microbial N supply in soil from the basalt and intermediate site. Therefore, the effect of the passage of soil through earthworms on microbial nutrient status depended on the nutrient deficiencies of the microflora and is assumed to vary among soil types and ecosystems. In earthworm casts deposited in soil from each of the study sites, additional P amendment did not result in an increase in microbial growth. This indicates that the additional P supply caused by the gut passage (cf. Sharpley

and Syers, 1977; Satchell and Martin, 1984; Scheu, 1987b) of the soils compensated microbial P deficiency. In contrast, the additional N supply caused by the gut passage (cf. Syers et al., 1979; Scheu, 1987c) did not eliminate microbial N deficiency completely. The effect of additional N amendment was similar in casts deposited in soil from each of the sites and increased AMR by factors of 1.4, 1.3 and 1.5.

MIRR was similar in earthworm casts and soil from each of the sites, indicating that existing microbial biomass in soils was affected only slightly by the gut passage through earthworms. Similar results were obtained in previous experiments investigating soils from different sites on limestone (Scheu, 1990).

The C_{org} content in soil from the limestone site exceeded that in soil from the basalt and intermediate site by a factor of about 2 (Table 1). Similarly, MIRR in limestone soil was about twice higher than in soil from the basalt and intermediate site. Insam and Domsch (1987) proposed the ratio between microbial biomass and soil carbon content (C_{mic}/C_{org}) as a measure of the stability of soil organic matter. Calculating C_{mic} from MIRR data according to the formula of Anderson and Domsch (1978) the ratios of C_{mic}/C_{org} in soil of the basalt, intermediate and limestone site were 0.013, 0.016 and 0.015 respectively. Therefore, a very similar amount of microbial biomass is sustained per unit C_{org} of the study sites indicating similar composition of the soil humus. A similar ratio of C_{mic}/C_{org} of 0.014 was found in a mull soil of a beechwood on limestone near Göttingen (Scheu, 1990). Presumably a ratio of C_{mic}/C_{org} of about 0.015 is typical for mature beechwood mull soils.

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The influence of soil compaction on microbial biomass and organic carbon turnover in micro- and macroaggregates

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ABSTRACT

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Agricultural practices with high traffic and with low input of organic material into the soil result in the deterioration of soil structure leading to soil compaction. It is accompanied by the reduction of gas exchange and by limited colonizable habitats. The changes in microbial biomass and its activity cannot be readily explained by interactions of altered gas and water exchange. In order to explain the differences in biological activity of compacted and uncompacted soils, the soil structure related microbial turnover was observed.

The content and the availability of soil organic carbon, microbial biomass and respiration activity in micro- (<0.2 mm) and macroaggregates (2–5 mm) from the plots with different wheel induced compactive treatments were measured in the layers 0–10, 10–20 and 20–30 cm. In the topsoil (0–30 cm) of the wheel tract plot the higher content of total carbon (0.942% C_{org}) and higher availability of C than in the no traffic (0.907% C_{org}) and high traffic (0.908% C_{org}) plots were established. The compacted plots contained less total carbohydrates in macroaggregates. The average value of microbial biomass was lower only in the high traffic plot — 99 µg C g⁻¹ against 159 in the no traffic plot. The compacted plots differed from the other treatments in the distribution of microbial biomass in soil layers; the maximum of microbial biomass was in the lower layers (20–30 cm) instead of in the surface layer. The “potential” activity of microbial biomass reached maximal values in the high traffic plot.

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INTRODUCTION

Soil structure, which is an important factor in agricultural land use, can deteriorate through compaction due to agricultural traffic. Compaction in this sense only means that a structural change can be expressed in terms of increasing soil bulk density. Intensive agricultural practices with high traffic result in soil structure deteriorations leading to compaction of soil layers (Raghavan et al., 1990).

The susceptibility of a given soil structure to compaction depends on the type of parent material, soil texture, organic matter content and moisture. A common feature of compacted layers is a reduction in the volume of larger pores and the absence of vertical biopores. The pore size distribution is shifted towards the fine pores ($<1\ \mu\text{m}$) (Manichon, 1987) and a decreased pore continuity and increased horizontal orientation is observed (Frede, 1987; Pagliai, 1987). This can limit growth and metabolism of biota in soil by a reduction of colonizable habitats (Kilbertus, 1980; Foster, 1988) and restrictions in gas and water exchange (Huysman et al., 1989; Kaiser et al., 1991). The latter authors observed in a field experiment with controlled agricultural traffic differences in microbial biomass and its metabolism between compacted and uncompacted plots, which cannot readily be explained by interactions of altered gas and water exchange. An additional explanation for the above observations might be found in soil structure related microbial turnover.

The mechanism of soil aggregation widely discussed in literature (e.g. Harris et al., 1966, Sequi, 1978) depends, to a large extent, on the quantity and metabolic activity of microorganisms (Lynch and Bragg, 1985). Soil aggregates consist of primary particles and various binding agents. The persistent binding agents are assumed to be responsible mainly for integrity of microaggregates, whereas macroaggregates are formed from microaggregates through the action of more easily decomposable binding substances as roots and filamentous microorganisms as well as plant and microbial polysaccharides (Tisdall and Oades, 1982).

In order to explain the changes in biological activity in compacted soils, we investigated the relations between carbon mineralization, microbial biomass and its "potential" activity in micro- and macroaggregates from plots with no agricultural traffic (NT), high traffic (HT) and from wheel tract (WT) that represent area with a high degree of compaction lasting practically throughout the year.

MATERIAL AND METHODS

Soil characteristics

The investigation was done with a loamy silt soil (pH 6.8, 10 mM CaCl_2) from a site at Braunschweig, Germany, where controlled agricultural traffic was applied since 1988 (Kaiser and Heinemeyer, 1990).

The common crop rotation of sugar beet, winter wheat and winter barley with conventional tillage is used at that site. In May 1991 soil of layers 0–10, 10–20 and 20–30 cm was sampled from three different winter barley plots (Table 1). Bulk densities were established in undisturbed samples from six different places. Soil for separation of aggregates was taken, in quantities of 5–7 kg, from two places and mixed together. The samples (average moisture 12.9% H₂O) were stored at 4°C for 7–14 days. Then the soil samples were dried (24 h, room temperature) in a moderate air flow.

Macroaggregates (2–5 mm) and microaggregates (<0.2 mm) were isolated by passing dry soil through 5, 2 and 0.2 mm sieves (10 s on automatic rotary shaker). For estimation of the relative amount of aggregate size fractions in the whole sample, 500 g samples were used ($n=3-5$).

Chemical analyses

The total carbohydrate content in soil (C_{ch}) was measured with the phenol-sulphuric acid procedure without prior hydrolysis (Šafařík and Šantrůčková, 1992). Briefly, 5 mg of crushed and sieved (<0.2 mm) soil diluted by 1 ml of water were mixed with 1 ml of 5% phenol solution, and 5 ml of concentrated sulphuric acid were added rapidly. The samples were blended on a vortex mixer for 10 s and allowed to stand at ambient temperature for 1 h; the absorbance of the supernatant was measured at 485 nm. The content of carbohydrates was expressed as glucose C.

The content of soil organic carbon (C_{org}) was established by dry combustion (induced furnace, Leco) after removing inorganic C (Nelson and Sommers, 1982).

Biological analyses

All soil samples were rewetted (50% WHC) immediately before analysing the biological activity.

The microbial biomass (C_{mic}) was estimated using the chloroform fumigation extraction method as described by Vance et al. (1987) and modified by Sparling and West (1989). The C content in soil extracts was determined by dichromate digestion without addition of HgO.

Metabolic quotients (q_{CO_2} , q_{O_2}) were calculated from C_{mic} and averaged CO₂ production and O₂ consumption rates measured in the first 10 h after rewetting in well aerated conditions using the automatic infrared gas analyser technique for CO₂ measurement (Heinemeyer et al., 1989) and BOD analyzer (Sapromat, Voith, Heidenheim, Germany) for O₂ measurement.

For mineralization measurements, CO₂ production was estimated after 1, 2, 4, 7, 12, 15 and 19 days of incubation at 28°C. Evolved CO₂ was trapped in NaOH; the excess NaOH was titrated by HCl after addition of BaCl₂. The

amount of carbon that became available by air drying, C_0 (mg C g^{-1}), the corresponding rate coefficient for its mineralization, k (day^{-1}) and the rate of carbon mineralization from the soil organic pool, C_B (mg C g^{-1}) were estimated by fitting the data for the measured mineralized carbon C_T (mg C g^{-1}) to a two component model of the form:

$$C_T = C_B t + C_0 (1 - e^{-kt})$$

using nonlinear regression (criterion: minimum sum of squares).

Statistical evaluation

The differences among measured characteristics were tested using analysis of variance (ANOVA). Significance of differences between the mean values of parameters estimated by the model were evaluated by *t*-test.

RESULTS

Physical characteristics (Table 1)

The 3 years regime of differential compactive treatments applied were reflected by the soil bulk densities and the aggregate size distribution. While the

TABLE 1

Soil bulk densities and aggregate size fractions in the topsoil (0–30 cm) of plots with different wheel-induced compactive treatments

Plot	Wheel passes (yr^{-1})	Layer (cm)	Bulk dens. (g cm^{-3})	Aggregate size fractions (mm)			
				< 0.2	0.2–2	2–5	> 5
No traffic	0	0–10	1.28 ± 0.05	28.4 ^{cd}	39.0 ^c	13.3 ^{ab}	19.4 ^{ab}
		10–20	1.30 ± 0.05	27.1 ^{bc}	39.7 ^c	14.6 ^{ab}	18.5 ^{ab}
		20–30	1.27 ± 0.03	33.9 ^c	38.3 ^c	15.3 ^b	13.4 ^a
High traffic	6	0–10	1.54 ± 0.06	31.1 ^{dc}	36.5 ^c	12.7 ^a	19.1 ^{ab}
		10–20	1.56 ± 0.11	22.8 ^a	39.3 ^c	15.3 ^b	22.7 ^b
		20–30	1.52 ± 0.06	24.9 ^{abc}	36.8 ^c	15.4 ^b	23.0 ^b
Wheel tract	20	0–10	1.69 ± 0.04	27.5 ^{bc}	32.4 ^b	15.9 ^b	24.1 ^b
		10–20	1.69 ± 0.03	23.8 ^{ab}	27.0 ^a	14.6 ^{ab}	34.7 ^c
		20–30	1.61 ± 0.04	24.5 ^{abc}	29.4 ^{ab}	15.6 ^b	30.5 ^c

Values within each column followed by different letters are significantly different at $p \leq 0.01$, ANOVA, $n = 3-5$).

TABLE 2

Contents of organic carbon (C_{org}), carbohydrate carbon (C_{ch}) and microbial carbon (C_{mic}) in micro- (<0.2 mm) and macroaggregates (2–5 mm) from 0–20 cm layers of plots with different wheel-induced compactive treatments

Plot	C_{org} (mg C g ⁻¹)	C_{ch} (mg C g ⁻¹)	C_{mic} (mg C g ⁻¹)	$C_{\text{mic}}/C_{\text{org}}$ (%)
<i>Microaggregates (< 0.2 mm)</i>				
NT	9.69 ± 0.17 ^a	2.14 ± 0.18 ^a	0.171 ± 0.007 ^a	1.8
HT	9.05 ± 0.11 ^b	2.14 ± 0.22 ^a	0.103 ± 0.021 ^c	1.1
WT	9.59 ± 0.40 ^a	2.19 ± 0.32 ^a	0.146 ± 0.042 ^b	1.5
<i>Macroaggregates (2–5 mm)</i>				
NT	9.76 ± 0.20 ^{ab}	3.78 ± 0.23 ^a	0.185 ± 0.036 ^a	1.9
HT	9.60 ± 0.34 ^a	3.18 ± 0.24 ^b	0.115 ± 0.017 ^b	1.2
WT	10.11 ± 0.37 ^b	2.27 ± 0.25 ^c	0.188 ± 0.012 ^a	1.9

NT = no traffic, HT = high traffic, WT = wheel tract.

Values within each column followed by different letters are significantly different at $p \leq 0.05$, ANOVA, $n = 6-10$).

relative amount of microaggregates (<0.2 mm) and small aggregates (0.2–2 mm) decreased, the relative amount of macroaggregates (2–5 mm) and larger particles as well as soil bulk densities increased with the frequency of wheel passes. The changes in the relative amount of microaggregates, small aggregates and larger particles were significant ($p \leq 0.05$, $n = 3-5$).

Organic carbon (Table 2)

In microaggregates, a significant ($p \leq 0.05$, $n = 6$) decrease in C_{org} was found for the HT plot. The carbohydrate content was uniform for all three plots and accounted for a range of 22.1–23.6% C_{org} . The microbial carbon and $C_{\text{mic}}/C_{\text{org}}$ ratios declined in the sequence NT > WT > HT.

In macroaggregates the C_{org} contents from the HT and NT plots did not differ but were significantly ($p \leq 0.05$, $n = 6$) lower compared to the WT plots. The carbohydrate content declined in the sequence NT > HT > WT. The carbohydrates of the NT and HT plots accounted for 38.7 and 33.1% of C_{org} , respectively, whereas in the WT plot only 22.4% were observed.

Microbial carbon contents and $C_{\text{mic}}/C_{\text{org}}$ ratios did not differ in NT and WT, but were significantly ($p \leq 0.05$, $n = 10$) lower in the HT macroaggregates.

Microbial activity and carbon mineralization (Tables 3 and 4)

“Potential” microbial activity measured in well aerated conditions after rewetting and expressed as metabolic quotients for CO_2 (q_{CO_2}) and O_2 (q_{O_2})

TABLE 3

Microbial activity expressed as metabolic quotients for CO₂ (q_{CO_2} ; $\mu\text{g CO}_2\text{-C mg}^{-1}\text{ C h}^{-1}$) and for O₂ (q_{O_2} ; $\mu\text{g O}_2\text{ mg}^{-1}\text{ C h}^{-1}$) of micro- (<0.2 mm) and macroaggregates (2–5 mm) from 0–20 cm layers of plots with different wheel-induced compactive treatments

Plot	Microaggregates		Macroaggregates	
	q_{CO_2}	q_{O_2}	q_{CO_2}	q_{O_2}
NT	2.5	0.53	2.3	0.49
HT	4.1	0.83	3.9	0.80
WT	3.2	0.64	2.7	0.73

NT=no traffic, HT=high traffic, WT=wheel tract.

TABLE 4

Total carbon mineralized during 19 days of incubation (C_T), the rate of mineralization of soil organic carbon pool (C_B), the amount of carbon released by drying (C_0) and mineralization rate coefficient of C_0 (k) of micro- (<0.2 mm) and macroaggregates (2–5 mm) from 0–20 cm layers of plots with different wheel-induced compactive treatments

Plot	C_T (mg C g ⁻¹)	C_B (mg C g ⁻¹)	C_0 (mg C g ⁻¹)	k (day ⁻¹)
<i>Microaggregates (<0.2 mm)</i>				
NT	194.1 ± 12.0 ^a	5.5 ± 0.22	92.8 ± 2.9	0.85 ± 0.07
HT	213.7 ± 9.5 ^a	5.7 ± 0.34	109.1 ± 4.7**	0.67 ± 0.06
WT	262.5 ± 19.9 ^b	7.0 ± 0.49*	132.1 ± 6.7**	0.71 ± 0.08
<i>Macroaggregates (2–5 mm)</i>				
NT	248.9 ± 17.2 ^b	7.0 ± 0.60	117.5 ± 7.8	0.51 ± 0.06
HT	313.4 ± 36.6 ^c	9.5 ± 0.76*	134.7 ± 10.9**	0.59 ± 0.09
WT	302.5 ± 15.5 ^c	8.7 ± 0.65*	138.1 ± 6.2**	0.52 ± 0.07

NT=no traffic, HT=high traffic, WT=wheel tract.

values of C_T followed by different letters are significantly different at $p \leq 0.01$, ANOVA, $n=8$ and values of C_B and C_0 followed by one or two asterixes are significantly different from NT at $p \leq 0.05$ or 0.01, t -test, $n=8$.

increased for both micro- and macroaggregates in the sequence NT < WT < HT (Table 3). The differences in q_{CO_2} and q_{O_2} between micro- and macroaggregates were small.

The carbon mineralized in a 19-day incubation period (C_T) was significantly ($p \leq 0.01$, $n=8$) higher in macroaggregates than in microaggregates in all cases. The rate of mineralization from the soil organic carbon pool (C_B) and the amount of carbon made available by drying (C_0), being calculated from a two component model, showed the same trend. The increased C_B and C_0 in macroaggregates, besides C_0 in WT macroaggregates, were significant ($p \leq 0.01$, $n=8$). The calculated mineralization rates for C_0 (k) were lower in

macroaggregates than in microaggregates. Differences were significant ($p \leq 0.05$, $n = 8$) in HT and WT soils.

Values of C_0 in microaggregates were significantly higher in HT and WT soils than in the NT soil. Values of C_T and C_B were higher only in the WT soil. In macroaggregates an equal trend compared to microaggregates was observed.

Distribution of carbon in the soil profile (Fig. 1)

Soil organic carbon (C_{org}) usually decreased with soil depth. The only exception was observed with macroaggregates from the HT plot and with microaggregates from the WT plot. When comparing the compactive treat-

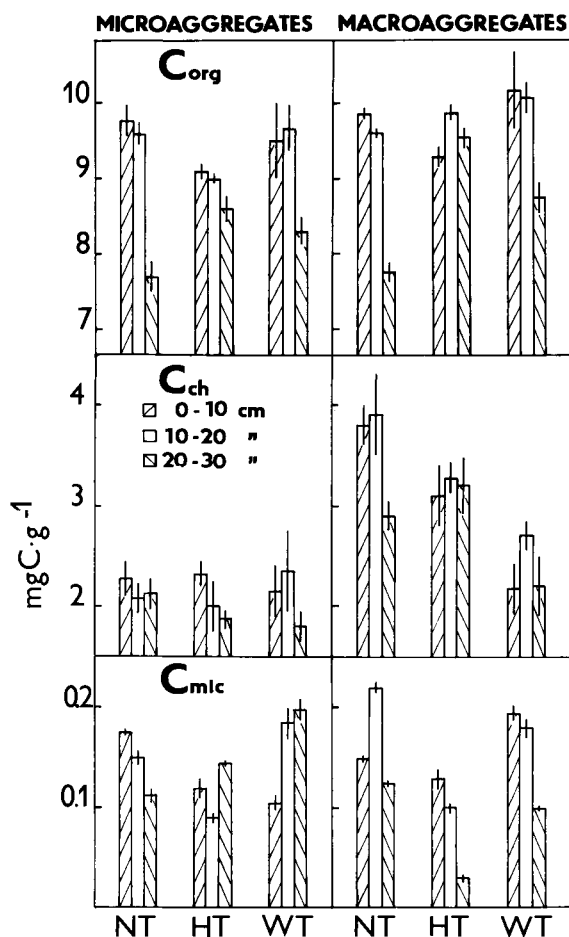


Fig. 1. Distribution of organic carbon (C_{org}), carbohydrates (C_{ch}) and microbial biomass (C_{mic}) in micro- (< 0.2 mm) and macroaggregates (2–5 mm) from soil layers 0–10, 10–20 and 20–30 cm. Mean values and standard deviation are given ($n = 3-5$).

ments, C_{org} in the 20–30 cm layer increased in the sequence NT < WT < HT plots in both aggregate sizes. The largest difference between macro- and microaggregates was found in the layer 10–20 cm of the HT plot, where C_{org} was significantly lower in microaggregates.

Soil carbohydrates in the 0–10 cm layer (C_{ch}) showed no general relation to deeper layers. Only the macroaggregates from the NT and HT plots had significantly higher C_{ch} contents, while the C_{ch} contents in all microaggregates and the WT macroaggregates were similar.

Microbial biomass carbon (C_{mic}) had the general trend to decrease with depth. An opposite trend was, however, found in microaggregates from the WT plot. Further exceptions were found in microaggregates from the HT plot, where the 20–30 cm layer had the highest C_{mic} content, and for macroaggregates from the NT plot, where the 10–20 cm layer had the highest C_{mic} content. Obviously, C_{mic} contents of the HT plot tend to be reduced in both aggregate sizes, while C_{mic} in NT and WT plots ranged similar.

DISCUSSION

The applied compactive treatments led to an increase of soil bulk densities and an increase of the relative amounts of largest aggregates which can reduce soil aeration (Table 1). The same observation was reported by Bakken et al. (1987). We wanted to elucidate in which way and to what extent these soil structural changes had an influence on soil microbial carbon turnover. Therefore we selected two fractions (<0.2 mm, microaggregates; 2–5 mm, macroaggregates) for further investigations.

With regard to the different compactive soil treatments, we found no correlation between resulted soil bulk densities and C_{mic} in both aggregate sizes studied (Tables 1 and 2). The HT plot showed the lowest values of C_{mic} , while the WT plot was not different from the NT plot though it received the highest compactive treatment. A possible explanation for this observation might be found in the duration of conditions with restricted aeration. For the WT plot these have been longer, since recompaction after ploughing was more rapid and complete than in the HT plot. A conservation effect for C_{mic} in extremely compacted soils (1.72 g cm^{-3}) was reported also by Anderson and Domsch (1989).

The decrease of C_{org} and C_{mic} established in the HT plot with slow gradual compaction after ploughing could also result from reaction of the microbial community on disruption of compacted layers. Soil tillage is always followed by a flush of organic matter decomposition, which is attributed to the crushing of soil crumbs, increasing aeration and the release of carbon (Carter, 1986). The disruption of compacted soil leads to more excessive changes of environmental conditions than the tillage of a well aerated soil. This was in-

licated by an increased mineralization rate after drying and sieving (Table 4).

We measured C_{mic} in dry soil and these results represent only the dormant and protected fraction of microbial biomass (Bottner, 1985). Although the soils were dried quickly without heating to reduce the changes of microbial biomass due to starvation, cell lysis and due to competition of bacteria and fungi (e.g. Rosacker and Kief, 1989), we have no information about the total microbial biomass. The low values of C_{mic} established in the HT plot could be explained by the low proportion of dormant fraction of microbial biomass in this soil while the total microbial biomass could be comparable to that of the NT and WT plots. Taking into account the low total microbial biomass in fresh samples from the same soils (Kaiser et al. 1991), we do not suppose this is the case. Assuming that C_{mic} calculated on the basis of C_{org} is an indicator for changes of soil biological properties (Insam et al., 1989) and of organic carbon content (Powlson and Brookes, 1987), the decrease of the C_{mic}/C_{org} ratio in micro- and macroaggregates of the HT plot and in microaggregates of the WT plot indicates that both soils, but especially the HT plot, are in the stadium of the loss of organic carbon and biological activity. The metabolic quotients q_{CO_2} and q_{O_2} were used as a criterion of microbial activity (Table 3). We established the quantity of microorganisms initially present in aggregates. Therefore, we had to know their respiration activity for calculation of the metabolic quotient. That is why we measured CO_2 production and O_2 consumption in well aerated condition in the first hours after rewetting of soil. The metabolic quotients calculated from these measurements have to be considered as "potential" characteristics. The high q_{CO_2} and q_{O_2} in the HT and WT plots showed a high capacity of microbial community to mineralize soil organic matter after improvement of environmental conditions of the compacted area that could lead to a decrease of organic carbon. However, we have no information about "actual" microbial activity in the compacted area. The limitation of it is probable but not proved. As CO_2 production was measured in air flow and O_2 consumption in a closed system, the respiration quotient RQ cannot be calculated from the data given in Table 3.

The differences were observed in carbon mineralization (Table 4). In the first days of incubation after rewetting, soil respiration increased exponentially when carbon, made available by the drying procedure, was utilized (C_0). After exhausting of this "additional" carbon, the respiration stabilized and was proportionate to the pool of carbon initially present in the soil (C_B). The mineralization rate coefficient (k) for C_0 depends on both the amount of microorganisms present and the quality of C_0 . A higher k -value indicates a lower mineralization rate. In microaggregates compared to macroaggregates, lower carbon contents and higher k -values were estimated. This supports the findings of Edwards and Bremner (1967) and Elliott (1986). They reported higher proportions of humified "highly processed" organic material in mi-

croaggregates compared to more labile organic matter in macroaggregates. Similarly Gupta and Germida (1988) reported lower mineralization of carbon in microaggregates than in macroaggregates.

Since carbohydrates (C_{ch}) mostly in the form of polysaccharides represent about one quarter of C_{org} and contribute towards soil aggregation (Oades, 1984), the effect of compaction on carbohydrate content was studied. Different compactive treatments showed an expressed negative influence on the C_{ch} content of macroaggregates, being lowest for the WT plot. Except for the WT soil, the carbohydrate content in macroaggregates exceeded that in microaggregates. The soil carbohydrates originate from plant and animal remains, from extracellular gums produced by microorganisms and from their cellular tissue (Cheshire, 1979). Turchenek and Oades (1979) found that microbe derived carbohydrates accumulated preferentially in the fine fractions and plant derived carbohydrates in the coarse fractions of soils. Therefore we suppose that carbohydrates of microbial origin prevail in microaggregates separated from investigated soils. The high content of carbohydrates in macroaggregates of the NT and HT soil can be attributed to the presence of roots, exudates and plant debris in various stage of decomposition. The WT plot contained practically no roots. However, a qualitative analysis of carbohydrates is necessary to verify this.

Our preliminary results indicate that soil compaction alone does not cause a decrease of microbial biomass and a decline in soil organic matter content. We propose that alternation of disruption and gradual recompaction leads to a decrease of soil organic matter and to loss of microbial biomass that is accompanied with an increase of specific microbial activity. For verification of this hypothesis, the investigation has to be completed by analyses concerning active microbial biomass, "actual" metabolic activity of microorganisms and the qualitative analyses of carbohydrates. For a better understanding information regarding aggregate densities and stabilities should be included.

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Immediate effect of wetting event on microbial biomass and carbohydrate production-mediated aggregation in desert soil

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ABSTRACT

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The interrelationship between microbial processes and soil physical structure following rapid wetting of dry soil was studied in a field experiment in the Negev desert. Short-term influence of four different amounts of water, 0, 5, 10 and 20 mm, applied in a single event, on microbial biomass, C-mineralization, degree of macroaggregation (> 0.3 mm) and aggregate stability was measured after 1 and 4 days. Immediately after wetting the amount of microbial biomass significantly increased coinciding with a decrease in C-mineralization rate; however, on the 4th day the biomass and the C-mineralization tended to stabilize again at the initial level of the non-irrigated control. Wetting of dry soil reduced the amount of carbohydrates within 4 days, regardless of water quantity added. More macroaggregates were formed in the wetted plots, probably due to physical attachment upon wetting cycle, but their stability was reduced compared to nonwetted aggregates. Carbohydrates were significantly correlated with aggregate stability, while microbial biomass and C-mineralization were not. Wetting of desert dry soil has triggered a rapid response in microbial activity-induced structure stability, and it was mostly dominated by the event itself, regardless of water quantity applied.

INTRODUCTION

Microbial processes play an important role in soil structure changes (Aspiras et al., 1971; Lynch, 1981). The relationships between the soil's biological and physical parameters are usually mediated by moisture availability, which is an important environmental variable governing microbial growth (e.g. Kushner, 1980). The carbohydrate fraction of soil organic matter, besides being a source of energy for microorganisms, forms complexes with metal

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ions and serves as an agent in binding inorganic soil particles into stable aggregates (Cheshire, 1979; Tisdall and Oades, 1982). Soil carbohydrates compose largely microbial extracellular polysaccharides (EPS) that are an immediate by-product of microbial carbon utilization. EPS have been assumed to be of great importance in ecophysiological processes regarding resistance to desiccation and survival in extreme environments (Wilkinson, 1958; Geesey, 1982). Haynes and Swift (1990) and Roberson et al., (1991), found that the soil structure and water infiltration rate were improved due to rapid and significant changes in the amount of carbohydrates in agricultural soil.

The role of soil biota in structure improvement of agricultural land in response to cultural practices is well recognized (Oades, 1984; Metting, 1986; Molope et al., 1987; Roberson et al., 1991). However, little is known about interrelations between microbial activity and soil physical parameters in desert lands. Moisture in deserts is relatively unavailable to biotas during most of the year. Noy-Meir (1973) has proposed that arriving "pulses" of such precipitation drive the basic route of energy flow and nutrient cycling in warm deserts. Since the amount, intensity and frequency of rainfall in the desert area varies greatly over time and space, the response of soil biota to such events is of great importance.

The present study aimed to examine the short-term effect of different amounts of water, applied in a single episode, on microbial process-mediated aggregation in the Negev desert soil.

MATERIALS AND METHODS

The experiment was conducted during the summer at the Avdat Research Farm for Runoff Agriculture, located in the northern Negev highlands of Israel (34°46'E–30°47'N). The area has a temperate desert climate with cool, wet winters (5–14°C range in January and 90 mm average winter annual rainfall) and hot, dry summers (18–32°C in June) with an annual evaporative demand of about 3000 mm. Dewfall occurring during approximately 200 nights amounts to 35 mm (Evenari et al., 1982). The soil is a deep, fine-textured loessial sierozem (Typic Calciorthids, Typic Camborthids).

Twelve square diked miniplots were divided into 4 quarters of 1 m² each by a cross-shape metal construction that had been carefully inserted to a depth of 20 cm in the previous year. The plots were kept bare (by hand weeding) during the study period. Four different irrigation treatments were randomly imposed in each miniplot (block), so that each quarter underwent one treatment. The treatments (with 3 replicates each) were of 5, 10, 20 and 0 (non-irrigated control) mm of water, respectively. The water was evenly applied in a single application, with a hand sprayer equipped with a T-jet No. 11012 nozzle (Spray System, USA). Soil temperatures, taken at noon, were recorded daily using soil thermometers installed at 5 cm depth in each quarter

(treatment) of one miniplot (block). Pooled soil samples (of approximately 200 g each), from 0–5 cm depth, were taken at the beginning of the experiment (before irrigation) and 1 and 4 days after irrigation treatments were imposed, put in plastic bags and immediately transferred to a 4°C cold room.

The following parameters were measured:

- (a) Gravimetric soil moisture content.
- (b) Microbial biomass, using the chloroform fumigation incubation method (Voroney and Paul, 1984).
- (c) Mineralization of carbon, by measuring CO₂ evolution of soil samples incubated for 20 days.
- (d) Natural carbohydrates were measured in acidic soil hydrolisates, using an extraction method similar to that of Oades et al. (1970) for extraction. The carbohydrates were determined with the anthrone method (Brink et al., 1960) with glucose as internal standard.
- (e) Degree of macroaggregation was carried out by passing the soils through 0.15, 0.30, 1.00, 2.00, 3.00 and 4.00 mm sieves. The mean weight diameter (MWD) was calculated according to Kemper and Rosenau (1986) and the degree of macroaggregation is the percent weight of aggregates > 0.3 mm in diameter.
- (f) Aggregate stability was estimated according to Roberson et al. (1991), using 5 g of air-dried macroaggregates (> 0.3 mm) placed in a 5 cm × 5 cm cylinder on a 0.3 mm sieve, covered by 2 layers of filter paper. Two liters of water were poured through and soil remaining on the sieve was oven-dried. Aggregate stability was expressed as the percent of oven-dry soil remaining on the sieve.

The last three parameters (d, e, f) were measured at the beginning (day 0) and at the end of the experiment (day 4).

All the data were subjected to analysis of variance (ANOVA) when significance was observed at the $p < 0.05$ level, Tukey's values were calculated for separation of means.

RESULTS

The addition of water to the dry desert soil led to an increase in soil water content (20 > 10 > 5 > 0 mm), followed by a lowering of soil temperatures (Fig. 1). After 4 days, soil temperature started to rise, the average water content decreased, and the data of soil moisture for the 5 and 10 mm amended plots were found to be identical (Fig. 1a). Still, the water content of the 20 mm treatment was twice the content of that of 5 and 10 mm. Soil temperatures did not respond according to the amount of water applied and at each sampling date they did not clearly differ (Fig. 1b). Microbial biomass had significantly ($p < 0.05$) responded on the first day after water addition. However, this immediate increase was reversed on day 4 when the average micro-

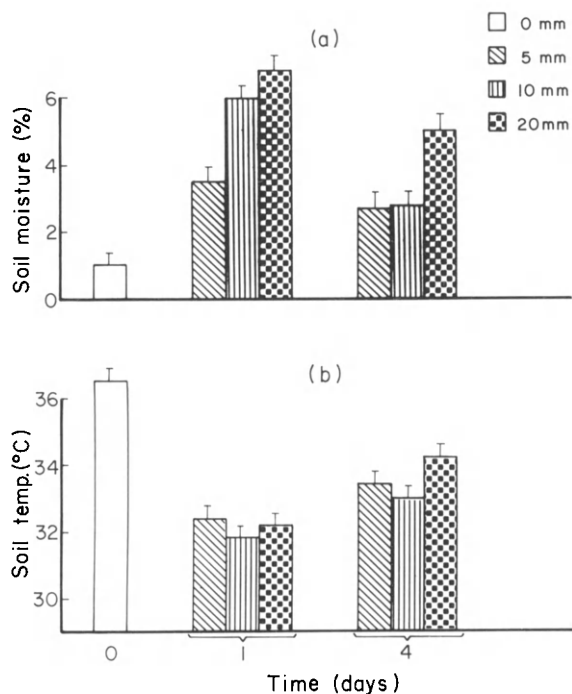


Fig. 1. Influence of water amendments on soil moisture (a) and soil temperature (b) during the study period (bars=S.D.).

bial biomass was equal to that of the nonirrigated soil (Fig. 2a). The rate of carbon mineralization decreased on the first day, and later on approximated the nonirrigated control treatment, although the average rates of the 3 sampling dates did not differ significantly due to high variance within treatments (Fig. 2b). The mineralization rate responded differently to water amounts at the end of the experiment, where the 5 mm treatment was significantly lower compared to that of 10 and 20 mm ($p < 0.05$) probably due to enhanced dryness. The amount of carbohydrate was significantly reduced at the end of the experiment due to irrigation, regardless of water amount (Fig. 2c).

The physical properties of the soil examined here generally showed opposite trends in response to irrigation. The weight percentage of aggregates of >0.3 mm (macroaggregates) significantly increased due to irrigation treatments (Table 1) and on day 4 it also could be seen in the mean weight diameter data. However, the percent of aggregate stability was lower in all irrigation treatments, compared to the nonirrigated control (Table 1), although the 20 mm treatment significantly differed from that of 5 mm of water.

The results of the correlation analysis are shown in Table 2. Carbohydrates were the only biological component that was highly correlated with aggregate stability. The inverse correlation with the degree of macroaggregation is prob-

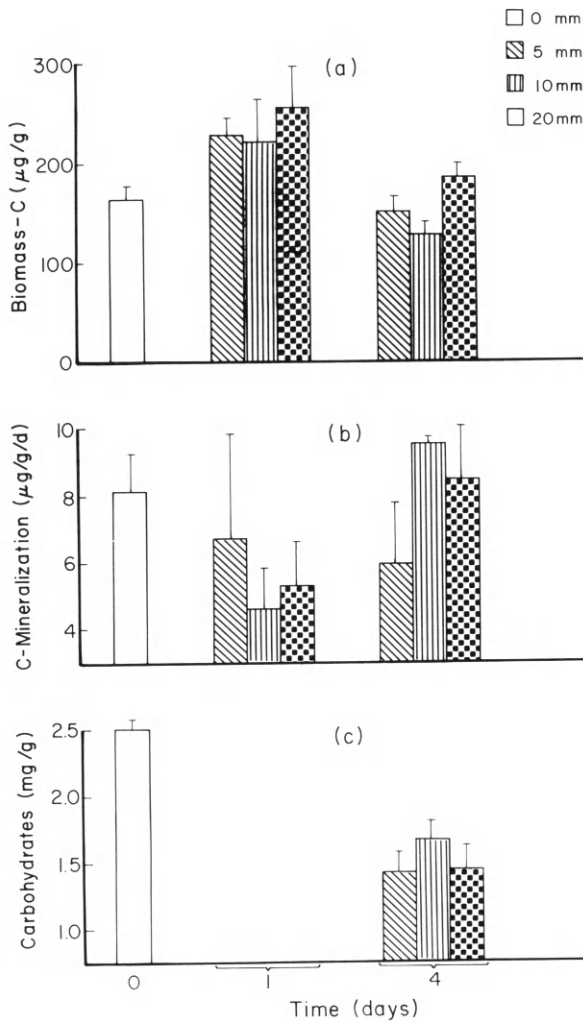


Fig. 2. Response of microbial biomass-C (a), C-mineralization rate (b) and soil carbohydrates (c) to wetting of desert dry soil (bars=S.D.).

ably caused by the dual effect of irrigation, i.e., on the one hand the decrease of carbohydrates and on the other the enhanced physical attachment of soil colloids.

DISCUSSION

The microbial processes examined responded immediately to the irrigation treatments; however, after four days it was found that microbial biomass and the rate of C-mineralization stabilized again at the basic levels similar to those

TABLE 1

Percent of macroaggregation, aggregate stability and mean weight diameter in response to water addition.

Treatment (mm)	Macroaggregation ^a (%)		Mean weight diameter (mm)		Aggregate stability ^b (%)	
	0	4	0	4	0	4
0	33	33 a ^c	0.62	0.62 a	63	63 c
5	–	44 b	–	0.85 bc	–	48 a
10	–	42 b	–	0.80 b	–	51 ab
20	–	48 c	–	0.87 c	–	53 b

^aAggregate of > 0.3 mm in diameter.
^bStability of air-dried macroaggregates.
^cWithin each column, numbers followed by the same letter are not significantly different at the *p* < 0.05 level.

TABLE 2

Correlation data for physical and microbial parameters of irrigated desert soil

	Aggregate stability	Macroaggregation	Carbohydrates	Biomass-C	C-mineralization
Aggregate stability	1				
Macroaggregation	0.27	1			
Carbohydrates	0.77**	–0.61*	1		
Biomass-C	0.18	0.26	0.40	1	
C-mineralization	0.43	0.14	0.50	0.22	1

***Significant at the 0.05 and 0.01 probability levels, respectively.

in the nonirrigated control. Wetting of dry soil is known to be followed by CO₂ flush, indicating intensive biological activity. Birch (1960) has reported that small showers and even dew formation can cause large increases in microbial populations, which are disproportionate to the amount of moisture added to the soil system.

Indeed, our data for microbial biomass are in agreement with the above, and emphasize mostly the effect of the wetting event rather than the quantity of water added. Since the desert ecosystem is a typically organic C-limited environment (Crawford and Gosz, 1982) a CO₂ flush in the case of sudden wetting of dry soil might be a result of mineralized intracellular solutes that are disposed after cell lysis caused by the increase in cell turgor potential, sometimes termed as “upshock” (Harris, 1981; Kieft et al., 1987). Nevertheless, we applied the fumigation method for biomass measurements and subtracted nonfumigated controls, in order to minimize the possibility of biomass overestimation due to biomass released by water potential increase

(Kieft et al., 1987). Moreover, we did not find significant differences between the amount of CO₂ produced by non irrigated soil at the beginning of the experiment (time "0"), compared to the CO₂ produced by nonfumigated irrigated soil during the experiment (regardless of water amount).

The immediate decrease in the rate of C-mineralization, which, however was not significant, can be explained as an indirect outcome of the wetting event. Even in the poor desert ecosystem moisture pulses might reveal and enhance the availability and utilization of some nutrient pools, especially nitrogen and organic carbon, by soil biota (Hadley and Szarek, 1981). In that case, the immobilization of carbon (and nitrogen) might be favored at the expense of mineralization. Later on, upon exhaustion of available nitrogen, the microbial processes of immobilization–mineralization will balance again at the basal level, which is typical for each ecosystem and also governed by climatic regime (Insam, 1990).

In considering the ecological function of microbial extracellular polysaccharides (EPS), Geesey (1982) mentioned that EPS is a film of nutrient acquisition which can promote, in some cases, nutrient accessibility to microbial cells. The assimilation of such "easy-quick" available sources by microbes and the exposure (and often loss) of organic matter due to soil perturbation can be reflected here by the reduction in the amount of carbohydrates on the 4th day. Probably they were consumed after the soil was irrigated, which may be supported by the rise in microbial biomass just after wetting. A rapid wetting of soil aggregates leads to physical–chemical processes of disintegration and dispersion. The later drying of soil colloids causes the shrinkage of the soil mass and leads to a mechanical cementation of particles (Taylor and Ashcroft, 1972). The higher percent of macroaggregates in the irrigated treatments in our experiment is in agreement with the above, but the stability of those aggregates was lower compared to the non-irrigated control, caused by consumption of the "easy-quick" carbohydrate as proposed above. The significant positive correlation between the amount of carbohydrates and aggregate stability points out that the formation and the stability of macroaggregates might be subjected to different mechanisms, and the carbohydrates (or EPS) contribute to the stabilization of already existing aggregates.

CONCLUSION

The experiment demonstrates the importance of carbohydrates as a stabilizing agent of soil aggregates in dry deserts. It shows that biological process-mediated soil structure is triggered, on a short-term scale, mainly by the wetting episode *per se*. It also suggests the existence of a well adapted microbial population that rapidly responds to environmental perturbation by exploiting "pulse reserves" for cell growth and activity.

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A microcosmic approach to compare effects of constant and varying temperature conditions on soil structure/soil biota interrelationships

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ABSTRACT

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A microcosm laboratory experiment was performed, modelling patterns of soil structure/biota interrelationships in a simple litter/sand ecosystem. The microcosms were populated by 3 different kinds of litter saprotrophic communities (microbiota; microbiota + *Nothrus silvestris* mites; microbiota + *Dendrobaena octaedra* worms) and kept at constant (15°C) and varying (10–20°C and 5–25°C) diurnal temperature regimes. After 4 months, organic matter loss due to respiration was similar in all treatments. However, the part of litter organic matter translocated by earthworms into a newly-formed “soil” horizon tended to correlate positively with a decrease in the range of temperature fluctuations. Earthworms were highly sensitive to the type of temperature regime, their mortality and cocoon production being maximal at constant temperature.

INTRODUCTION

Temperature is one of the most important factors determining levels of biota metabolic activity, as well as rates of decomposition processes in soil (Swift et al., 1979). Responses of soil ecosystems to different temperature conditions affecting the processes of organic matter transformation as well as interrelationships within soil communities, are widely discussed in literature. However, the effects of stable temperature regimes have mainly been compared, and analysis of the reactions to natural temperature fluctuations has

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somehow remained outside consideration. As exceptions should be regarded publications devoted to the problem of winter soil activities, and to the importance of successive freezings and thawings for changes in the structure of plant remains and acceleration of decomposition and metabolic reactions of soil microflora (e.g. Hurst et al., 1985; Taylor and Parkinson, 1988a; Skogland et al., 1988; Allen-Morley and Coleman, 1989). It is still an open question as to which extent processes and relationships in a soil ecosystem can be determined by regimes of diurnal temperature oscillations within the limits optimal for the functioning of soil biota. In this paper a methodical approach on the basis of laboratory microcosms is developed to compare these effects in decomposing forest litter populated by different kinds of saprotrophic communities.

EXPERIMENTAL DESIGN

Experimental microcosms represented 150 ml plastic containers filled with sand (65.5 ± 0.5 g) covered with a mixed birch/oak leaf litter (2.5 ± 0.2 g), each layer occupying approximately 1/3 of the container's volume. The litter was collected from the F horizon of a birch/oak (with pine) forest in Kampinos National Park, Warsaw region. Both substrates were thermically sterilized at 105°C during 24 h. The leaf material was slightly fragmentated (to pieces of 2–3 cm), twigs removed, to create, as much as possible, initially identical microcosms.

Microflora was inoculated into microcosms with a water suspension prepared from the same litter. Two kg of intact litter were soaked in distilled water for 3 h, and the suspension was squeezed out through a 1 mm and subsequently 0.1 mm mesh sieve. Twenty ml of the suspension (containing 40.4 ± 1.3 mg of organic matter) were added to the dry litter of each microcosm.

Three series of microcosms were organized, inhabited by different kinds of saprotrophic communities: (1) microbiota, represented by microflora and microfauna (Protozoa, Nematoda, Rotatoria, Tardigrada) populations, having been introduced into the litter with the suspension; (2) microbiota + 50 specimens of a panphytophagous mite (*Nothrus silvestris* Nicolet, *Oribatei*); (3) microbiota + 1 adult worm of *Dendrobaena octaedra* (Savigny) (*Lumbricidae*). Both, *N. silvestris* and *D. octaedra*, were common dwellers of the birch/oak litter of the Kampinos National Park.

The microcosms were distributed over 3 climatic chambers of HERAEUS-Votsch (HPS-1500) type, modelling different patterns of diurnal temperature regime: (1) 15°C constantly; (2) oscillations from 10°C ("night") to 20°C ("day"); (3) from 5°C to 25°C at "night" and "day", respectively. The range of temperatures in the litter of the 2nd variant can be considered normal for the natural habitat in summer, though the changes proceeded somewhat faster

in the chambers (for about 1 and 1.5 h in the 2nd and 3rd variants, respectively). "Day" conditions (12 h) were also indicated by weak illumination of about 5 kLux at the top surface of the microcosms. Air humidity in the chambers and water content of the substrates (litter + sand) were maintained constant at the level of 80% and ca. 75% of the microcosm's holding capacity, respectively. Following checks of the dynamics of the latter parameter (in a special series of microcosms) and evaporation rate (by individual weighing of the containers), water loss was compensated twice a week. Lids made of 0.1 mm nylon mesh prevented the substrates from rapid water loss, as well as from the escape of invertebrates. Each chamber contained ca. 150 microcosms representing all the 3 biota series.

The experiment lasted from the end of May till October, 1991, and samplings were carried out monthly. Data below are presented for one sampling occasion only. At sampling dates, 4 to 8 microcosms per series in each chamber were analyzed in the following sequence: (1) measurements of "day" and "night" CO₂ production by the whole microcosms and (in earthworm series) by the worms; (2) estimations of wet and (after fauna extractions and earthworms' cocoons counting) dry weight of microcosm's fractions (litter, sand and, in earthworm series, newly-formed "soil"); (3) evaluations of organic matter content (loss by ignition; used as the index below) and its elemental composition in the fractions.

PRELIMINARY RESULTS AND CONCLUSIONS

Detailed results and discussions are being prepared to be published in a series of papers elsewhere. The preliminary considerations below merely serve for testing the approach proposed and show possible ways of interpreting the data obtained.

At the start of the experiment, microcosms contained 2.4–2.7 g of organic matter (2.0–2.3 and 0.37–0.38 g in litter and sand layers, respectively). After 4 months of decomposition, no significant differences (according to the Student–Newman–Keuls multiple range test, after Zar, 1984) between any of the biota series or temperature variants were found in the proportions of the organic matter disappeared from the whole microcosm ecosystems (including litter and sand layers) (Table 1, A). This parameter represents the part of the microcosms' initial organic matter having been lost due to community respiratory metabolism.

On the other hand, organic matter disappearance from the litter layer was similar for the series of microbiota and microbiota + mites, whereas in the earthworm treatments it was significantly higher (Table 1, B). Contrary to the data of the 1st and 2nd series, earthworms' feeding and burrowing activity at all the temperature regimes led to evident changes in the structure of microcosms (Fig. 1). As a result, a new "soil" fraction had developed between

TABLE 1

Organic matter loss (% of the initial amount) in the whole microcosm ecosystem (A) and in the litter layer (B), as influenced by different saprotrophic communities and temperature regimes

Index	t^0 regime (°C)	M	M+N.s.	M+D.o.
A	15	20.3 (1.8)	21.8 (1.8)	21.1 (0.8)
	10-20	19.8 (2.1)	19.5 (1.5)	19.1 (1.2)
	5-25	20.1 (1.4)	19.1 (1.8)	24.6 (2.7)
B	15	21.2 (1.2)	22.1 (1.1)	65.3 (1.3)
	10-20	21.7 (1.3)	22.4 (0.7)	58.9 (3.3)
	5-25	20.9 (0.3)	20.9 (1.0)	59.5 (3.8)

S.E. in parentheses, 5 to 6 replicates per each data. Series of the experiment: M=microbiota; M+N.s.=microbiota+mites; M+D.o.=microbiota+earthworms.

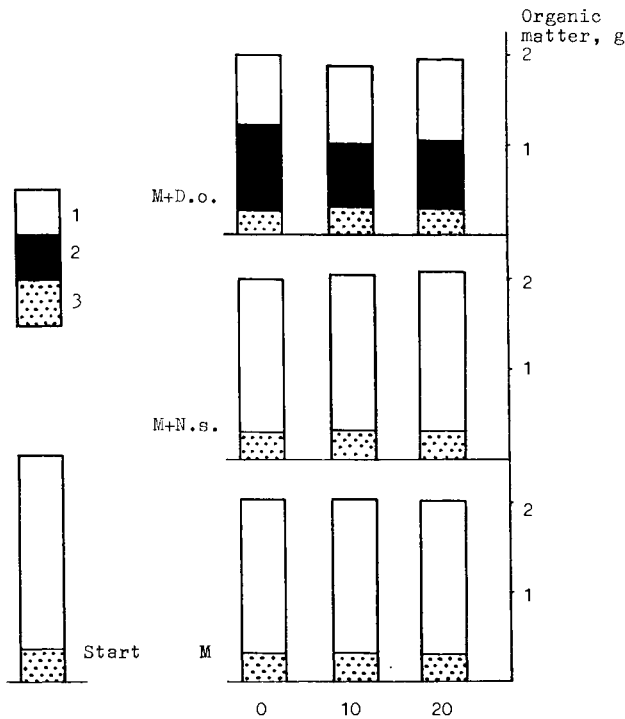


Fig. 1. Changes in the structure of the microcosms after 4-month-long decomposition, as compared with the one at the initial moment (*Start*): average amounts (n from 5 to 6) of the organic matter (g) in litter (1), newly-formed "soil" (2) and sand (3) layers. *M*, *M+N.s.*, and *M+D.o.*=experimental series of microbiota, microbiota + mites, and microbiota + earthworms. X-axis (0, 10, 20)=amplitude of temperature fluctuations (°C).

TABLE 2

Ratios between the amount of the organic matter translocated by the earthworms and (A) its initial amount in the microcosms ($\times 100\%$); (B) the amount attributed to the microcosms' respiration

t^0 regime ($^{\circ}\text{C}$)	n	A	B
15	6	38.3	1.82
10-20	6	33.3	1.74
5-25	5	29.4	1.20

S.E. in parentheses, n = number of replications.

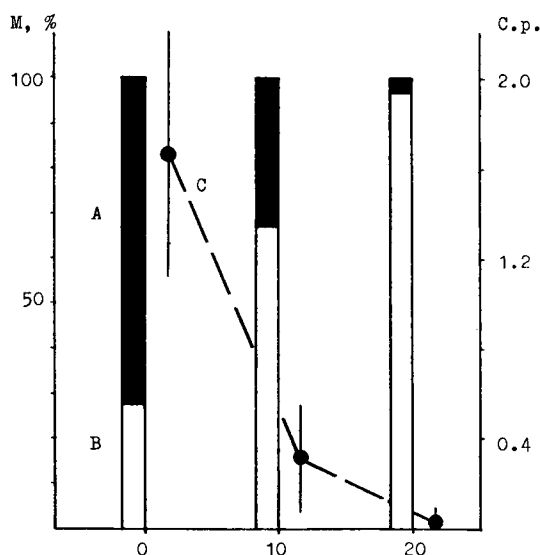


Fig. 2. Earthworm average mortality (M, %; A = worms died, B = worms survived) and rate of cocoon production (C.p., cocoons worm $^{-1}$ month $^{-1}$, $\pm 95\%$ confidence intervals) (C) under different temperature conditions, for the last 2 months and the whole of the experiment, respectively. Each point is based on 23-24 (M) and 38-44 (C.p.) microcosms. X-axis (0, 10, 20) = amplitude of temperature fluctuations ($^{\circ}\text{C}$).

the litter and sand layers, consisting of small litter and sand particles, but mainly of earthworm faeces. The amount of the organic matter in this fraction can be regarded as a measure of its translocation within the microcosms due to the functioning of *D. octaedra*.

Comparing the values of this parameter with the initial organic matter amount in the microcosms, a negative correlation was revealed between the relative importance of the translocation process and the range of diurnal temperature fluctuations (Table 2, A). A similar pattern was obtained when comparing the values of the microcosms' organic matter translocated and res-

pired: their ratio (being always over 1) markedly dropped between the variants of 15°C and 5–25°C (Table 2, B).

In turn, life strategies of *D. octaedra* were drastically influenced by the character of the environmental conditions. Correlating with the narrowing amplitude of temperature fluctuations, the level of worm mortality sharply increased, but so did the rate of cocoon production (Fig. 2). These effects were mainly observed during the last 2 months of the experiment. Under the regime of 5–25°C, only 1 worm died (at the end of the experiment), and practically no cocoons appeared.

Thus, the character of temperature regime had provided certain effects on the transformation of organic matter in laboratory microcosms, at least in the presence of earthworms, who themselves turned out to be greatly sensitive to the influence of that factor.

It should be added that, as far as I know, direct information in literature on the topic discussed is practically absent. Concerning effects of temperature conditions on decomposition processes or earthworm activities, the data are restricted to seasonal changes or comparisons of stable temperature regimes (Swift et al., 1979; Lee, 1985; Taylor and Parkinson, 1988b, c; and many others), or freezing/thawing perturbations (Taylor, Parkinson, 1988a; Allen-Morley, Coleman, 1989). A more or less marked decrease in the rate of cocoon production under fluctuating temperatures comparing to constant ones of 25, 20 and 15°C (but not of 10°C), had been observed in short-term experiments for *Eisenia foetida* (Reinecke, Kriel, 1981), thus being in agreement with the results obtained here. Several publications on the same species (reviewed by Lofs-Holmin, 1985) reported a slight rise in the number of hatchlings per cocoon under fluctuating vs. constant temperatures.

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Microbial biomass and activity in soils with fluctuating water contents

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ABSTRACT

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Two soils (an Alfisol from Borgloon, Belgium, and a Spodosol from Meer, Belgium) were amended with ^{14}C -labelled residues of maize plants and incubated for 7 days (Borgloon soil only) and 27 days (both soils). Samples from each soil were then subjected to either (1) drying at 40°C for 3 days, remoistening and incubation at 25°C for 10 days, or (2) storage at 4°C for 3 days and incubation. During drying or storage and subsequent incubation of soils, biomass C and ^{14}C were determined using a fumigation extraction method. Amounts of $^{12}\text{CO}_2$ and $^{14}\text{CO}_2$ evolved during the 10-day incubation period were monitored.

Drying and rewetting of soils caused a decrease in microbial biomass and enhanced mineralization of carbon during subsequent moist incubation. During drying unlabelled biomass C declined to 70–82% of control soils. Labelled biomass C decreased relatively more by soil drying and rewetting than unlabelled biomass C. Largest relative decreases of biomass ^{14}C caused by soil drying and rewetting were observed when biomass ^{14}C showed the highest specific respiration activity. After 7 days of incubating Borgloon soil with labelled plant material, soil desiccation and remoistening decreased biomass ^{14}C to 37% of control soils. When this soil was subjected to drying after 27 days of incubation the relative decline in biomass ^{14}C was smaller, viz. to 60% of control soils. Corresponding respiration activities were 30 and $8\ \mu\text{g C mg}^{-1}\text{ biomass C day}^{-1}$ after incubating Borgloon soil for 7 and 27 days, respectively. After 27 days of incubation with labelled plant material, biomass ^{14}C of Meer soil decreased relatively more than biomass ^{14}C of Borgloon soil, previously incubated for 27 days. Specific respiration activity for biomass ^{14}C of Meer soil was $16\ \mu\text{g mg}^{-1}\text{ biomass C day}^{-1}$. It was concluded that the resistance of soil microorganisms to soil drying and rewetting is influenced by their metabolic activity (which is determined by their type and physiological state). Other soil-related factors are not excluded, but appeared to be of minor importance only.

The sources of enhanced mineralization after drying and rewetting are organic substrates derived from microorganisms killed by drying and, to a larger extent, from other non-living soil organic matter. The contribution of the non-biomass soil organic carbon to the mineralization flush after soil drying and remoistening is largely dependent on the localization of microorganisms in the soil structure relative to their substrates. Studying the effects of soil drying and remoistening is proposed as a possible way to unravel those important consumer–substrate relationships.

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INTRODUCTION

Drying and remoistening of soils influences profoundly microbial biomass and activity (Lund and Goksøyr, 1980; Bottner, 1985; Kieft et al., 1987). Increased amounts of C and N are mineralized during incubation of dried and rewetted soils, when compared with mineralization of soils that are kept moist (Soulides and Allison, 1961; Sørensen, 1974). The sources of these mineralization flushes are decomposable organic substrates which are partially derived from the death of a portion of soil microorganisms, and partially from non-living soil organic matter (Jenkinson, 1966; Sørensen, 1974).

In a previous study (Van Gestel et al., 1991), we observed that similar proportions of biomass disappeared by drying in soils of contrasting texture and aggregate stability, which suggests that factors other than soil characteristics may determine the decline in microbial biomass by drying. Here we examine the effect of drying and rewetting on microbial communities in the same soil but with different metabolic activities, which are expressed by specific respiration activities.

MATERIALS AND METHODS

Soils

Two soils from a temperate humid climate region (near Borgloon and Meer, Belgium) were sampled from the 0–10 cm depth layer of agricultural farm land.

The Borgloon soil was an Alfisol (typic hapludalf) of pH 6.9 and contained 0.39% N and 3.8% C. Of the organic carbon, 1.9% ($741 \mu\text{g C g}^{-1}$ soil) was accounted for as biomass C. This soil was a silty loam with a clay content of 16.5%.

The Meer soil was a Spodosol (typic haplorthod) of pH 5.2 and contained 0.12% N and 1.5% C, of which 0.9% ($142 \mu\text{g C g}^{-1}$ soil) was biomass C. The Spodosol was a loamy sand and contained 9% clay.

Incubation of soils amended with ^{14}C -labelled plant material

Field-moist Borgloon and Meer soils were partially air dried, sieved (3 mm) and stored at 4°C before use. The soil water content was adjusted to 40% of waterholding capacity, and the soils were incubated at 25°C for one week before amendment with ^{14}C -labelled plant material.

Replicate samples of moist soils (equivalent to 30.0 g of oven-dry soil) were weighed into glass beakers, to which were added 30.0 mg of ground, ^{14}C -labelled maize plants (*Zea mays*) (%C=37.4; %N=0.94; specific activity=4.8 MBq g⁻¹; Vanlauwe et al., unpubl.). After thoroughly mixing of plant

and soil, beakers were put in pairs in 1.5 l glass jars containing distilled water and a test tube with 10 ml 1N NaOH to absorb evolved CO₂. Jars were closed with glass lids, rubber bands and clips, and incubated at 25°C. After 11 days of incubation, CO₂ traps were renewed.

Drying, rewetting and incubation of soils

After 7 days of incubation (for Borgloon soil only), and after 27 days of incubation (for both soils), replicate samples were used for the determination of biomass C and biomass ¹⁴C concentrations. Remaining samples were either (1) dried at 40°C for 3 days, then remoistened with distilled water to their respective original moisture contents (40% of WHC), or (2) stored for 3 days in a cold room at 4°C (to minimize microbial activity). These latter samples became the control soils for determination of the influences of soil drying. After 3 days of drying the water potential of each soil exceeded $-1.18 \cdot 10^3$ MPa.

All soil samples were put in sealed 1.5 l jars containing a vessel with 10 ml of 1N NaOH to absorb evolved CO₂, and were incubated at 25°C for 10 days. At specified times during drying or storage of soils, and during their subsequent incubation, samples were removed for analyses of biomass C and ¹⁴C. These times were: at day 1 and 3 during soil drying; after 1, 2 and 10 days of incubation of dried and remoistened soil samples; after 3 days of storage at 4°C of control soils; and after 2 and 10 days of incubation at 25°C of control soils. Evolved CO₂ was determined at the same times. All analyses were carried out in triplicate.

Analyses

Biomass C and ¹⁴C contents were determined after fumigation of soils with ethanol-free CHCl₃ vapour for 10 days at 25°C, and extraction with 2N KCl. The amounts of ninhydrin-reactive N of filtered (Millipore membrane, 0.45 µm) extracts were determined from absorbances at 570 nm after reacting 0.5 ml aliquots with reagent (2.0 g of ninhydrin and 0.2 g of hydrindantin dissolved in 1:4 mixture of 4N sodium acetate buffer and dimethyl sulfoxide). Total biomass C was estimated from the gain in ninhydrin-reactive N after fumigation, multiplied by 21 (Amato and Ladd, 1988). The radioactivity of soluble ¹⁴C was determined on 1.0 ml aliquots of filtered extracts mixed with scintillation cocktail. Biomass ¹⁴C was estimated from the gain in extractable ¹⁴C multiplied by 3.25 (Ladd and Amato, 1988). Biomass ¹²C contents were obtained by subtraction of biomass ¹⁴C from total biomass C values.

Total CO₂ absorbed in alkali was determined by titration with 0.05N HCl (Tinsley et al., 1951). The radioactivity of absorbed ¹⁴CO₂ was measured using 0.2 ml aliquots of alkali mixed with 10 ml of scintillation cocktail.

RESULTS AND DISCUSSION

Biomass C and ^{14}C during decomposition of added plant material

After 7 days of incubation of the Borgloon soil, 45.8% of the ^{14}C added as labelled plant material was released as $^{14}\text{CO}_2$; and 11.2% of the total microbial biomass C consisted of labelled biomass C, corresponding to $102.1 \mu\text{g C g}^{-1}$ soil (Table 1). After 27 days of incubation, 55.5% of added plant carbon had evolved from the Borgloon soil. Biomass ^{14}C content was then $81.6 \mu\text{g C g}^{-1}$ soil or 9.3% of total biomass C.

Decomposition was slightly faster in the Meer soil, where 58.3% of plant input ^{14}C was transformed into $^{14}\text{CO}_2$ after 27 days of incubation. Corresponding first-order net decay rates, averaged over the period 11–27 days, were 0.009 and 0.012 day^{-1} for the Borgloon and the Meer soil, respectively. After 27 days of incubation, the biomass ^{14}C content of the Meer soil was lower than for the Borgloon soil and amounted to $60.1 \mu\text{g C g}^{-1}$ soil or 27.6% of total biomass C, in agreement with Gregorich et al. (1991) who reported that lower concentrations of biomass C were maintained in soils with lower clay contents.

Respiration activity of biomass ^{14}C

The specific respiration rate ($q\text{CO}_2$) (CO_2 respired per unit of biomass C) is considered to reflect the metabolic activity of the microflora. “Young” cells and populations composed of mainly r-strategy ecotypes have typically a higher specific respiration than “older” cells or K-strategists (Insam, 1990; Insam et al., 1991). The metabolic quotients ($q\text{CO}_2$) of labelled biomass before commencement of treatments were calculated from amounts of $^{14}\text{CO}_2$ evolved from stored control soils during the 10-day incubation period (Table 1).

The metabolic activity of microbial biomass (and the specific respiration

TABLE 1

Percentage of added ^{14}C -labelled plant material decomposed, biomass C and ^{14}C concentrations, and specific respiration activity of biomass ^{14}C

Soil	Incubation time (days)	% of added plant material decomposed	Biomass C ($\mu\text{g C g}^{-1}$ soil)	Biomass ^{14}C ($\mu\text{g C g}^{-1}$ soil)	Specific respiration activity of biomass ^{14}C ^a ($\mu\text{g C mg}^{-1}$ biomass C day^{-1})
Borgloon	7	45.8	914(8) ^b	102.1(3.1)	30
Borgloon	27	55.5	877(15)	81.6(3.3)	8
Meer	27	58.3	218(11)	60.1(2.3)	16

^a $^{14}\text{CO}_2$ evolved from control soils during the 10-day incubation period per unit of biomass ^{14}C .

^bStandard deviation in paratheses.

rate) is high when large amounts of substrate are available, or when the turnover of biomass C is fast (with accompanying low biomass levels). For the Borgloon soil, the specific respiration of biomass ^{14}C present after 7 days of incubation with labelled plant material, was 3–4 times higher than when incubated for 27 days, reflecting the higher available substrate concentration after 7 days of incubation. After 27 days of incubation, the respiration activity of biomass ^{14}C content of the Meer soil was twice that of the Borgloon soil (Table 1). Because available substrate concentrations were similar for both soils at that time (added plant material was decomposed to a similar extent), the difference in metabolic activity must be due to a difference in turnover of labelled biomass between both soils. For the sandy Meer soil, biomass ^{14}C content was lower and the turnover of biomass ^{14}C higher than for the loamy Borgloon soil.

Corresponding specific respiration rate of biomass ^{12}C was $22 \mu\text{g C mg}^{-1}$ biomass C day^{-1} for the Borgloon soil after 7 days of incubation, and 14 and $34 \mu\text{g C mg}^{-1}$ biomass C day^{-1} for the Borgloon and Meer soil (incubated for 27 days) respectively.

Decline of microbial biomass during drying

Labelled and unlabelled biomass C declined during desiccation and increased rapidly on rewetting and further incubation to values which remained below those of control soils (Fig. 1). For all three cases, biomass ^{14}C declined relatively more than biomass ^{12}C (Fig. 2). Biomass ^{14}C of the Borgloon soil incubated for 27 days decreased to 60.2% of biomass ^{14}C content of control soil after drying and rewetting. A larger decrease, viz. to 37.4% of control soils, was observed when this soil was subjected to desiccation and remoistening after 7 days of incubation with ^{14}C -labelled plant residues.

Types of microorganisms which are able to respond quickly to an available carbon source (r-strategists) will proliferate more than the slower growing K-strategist cell types after the addition of ^{14}C -labelled plant material to soils. Thus, relatively more biomass ^{14}C will be associated with active organisms following r-strategies of growth, than with K-strategists. When amounts of readily decomposable carbon had declined, rates of decomposition of added plant material also decreased, as well as the metabolic activity of soil microorganisms (Bottner, 1985). Consequently, biomass ^{14}C determined after 27 days of incubating residue-amended Borgloon soil, consisted, on average, of lower proportions of cells with a high metabolic activity than biomass ^{14}C assayed after 7 days of incubation. The larger decrease of biomass ^{14}C by drying and rewetting of soils after 7 days than after 27 days of incubation, suggests that cells with a high metabolic activity are more susceptible to drying than less active microorganisms.

Biomass ^{14}C present in the Meer soil after 27 days of incubation decreased

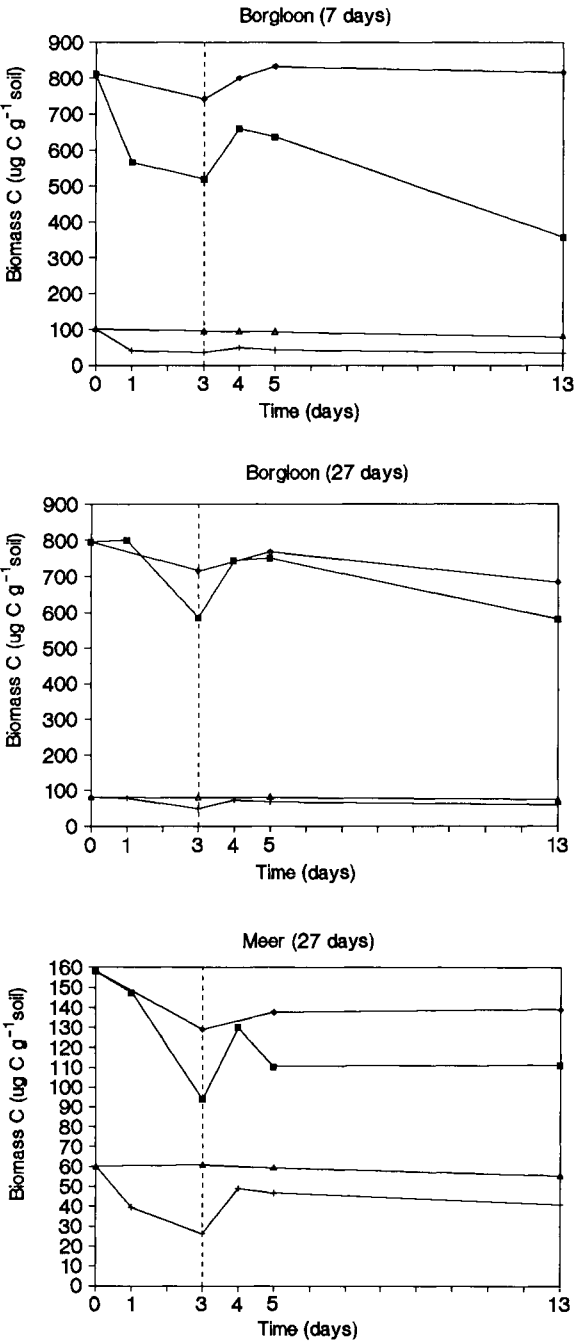


Fig. 1. Effect of drying (+, ■) or storage (△, ◆), and subsequent incubation on labelled (+, △) and unlabelled (■, ◆) biomass C contents of Borgloon soil, incubated for 7 days and 27 days prior to treatments, and of Meer soil, incubated for 27 days with ¹⁴C-labelled plant material before commencement of treatments.

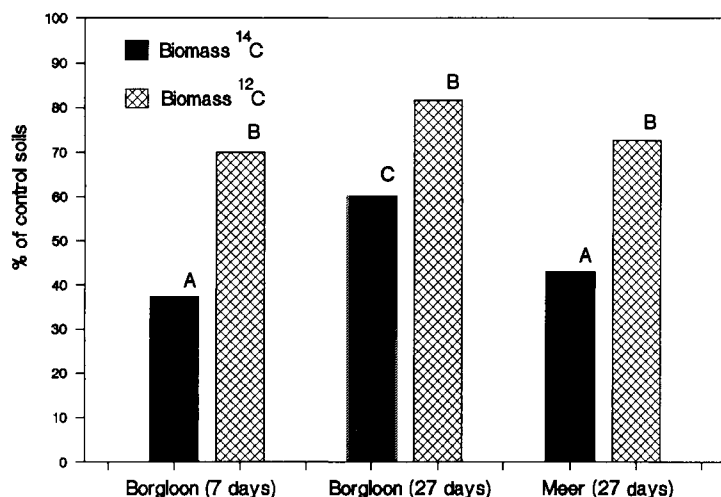


Fig. 2. Labelled and unlabelled biomass C after drying, expressed as percentage of biomass C contents of stored control soils. Bars with the same symbol were not significantly different at the 5% level.

also more by soil desiccation and remoistening than was observed for the biomass ^{14}C content of the Borgloon soil previously incubated for 27 days (to 43.0% of control soil) (Fig. 2). This supports the hypothesis that microbial resistance to soil desiccation is influenced by their metabolic activity, which is dependent on their type or physiological state. This conclusion is corroborated by other studies reporting that slow-growing microorganisms were more resistant to soil drying than fast-growing types (Boylen, 1973; Bushby and Marshall, 1977).

Further, in a previous study (Van Gestel et al., 1991) we observed that similar proportions of biomass disappeared by drying in soils of contrasting texture and aggregate stability, suggesting that properties of the microbial population are the more important factor determining microbial survival during soil desiccation. West et al. (1992) reported similar conclusions. Nevertheless other authors have stated that soil-related factors such as adsorption of clay particles (Marshall, 1975) or location in the soil fabric (Hattori, 1973, 1988) influence the survival of microorganisms during soil drying. This does not necessarily contradict the above-mentioned conclusion because fast-growing organisms decomposing added substrates, are most probably predominantly located on the outside of microaggregates, and are not yet surrounded by thick clay coatings. Both factors, one based on microbial properties and the other on soil characteristics, may be at work simultaneously.

Increased mineralization after soil drying and rewetting

Soil drying and rewetting increased amounts of C mineralized during subsequent incubation. Figure 3 shows percentages of biomass C before soil

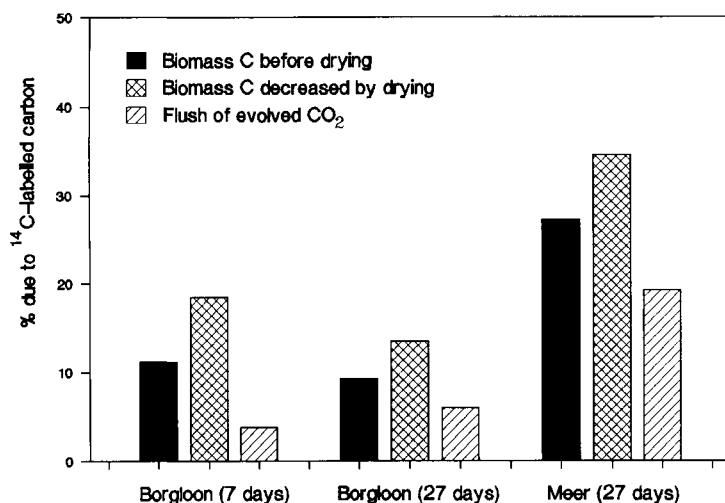


Fig. 3. Percentage due to plant-derived, ¹⁴C-labelled carbon, of biomass C before soil drying, of biomass C decline by drying, and of flushes of CO₂ from dried soils after incubation and correction for release from stored control soils.

drying, of biomass C declining by drying, and of CO₂ flushes due to labelled plant-derived carbon. The fraction of total C mineralization flush due to ¹⁴C-labelled plant material was always lower than for biomass C before treatments, or for biomass C killed by drying (Fig. 3). This indicates that drying and rewetting also made organic components available for mineralization from non-living soil organic matter.

Drying and rewetting of soils causes physical disruption of the soil structure, substrate desorption from soil surfaces, and increased microbial mobility and diffusion of soluble organic compounds (Jenkinson and Powlson, 1976; Lund and Goksøyr, 1980; Kieft et al., 1987). Thus the location of microorganisms in relation to their substrates is altered, promoting mineralization of C from sources which were not available for decomposition before soil drying.

The size of the contribution of non-biomass soil organic matter to the flush of mineralization is influenced by the concentration and quality of soil organic carbon and by soil characteristics such as texture and microporosity. This explains the larger relative difference between the specific activity of microbial C and of the CO₂ flush after drying and rewetting for the Borgloon soil than for the Meer soil (Fig. 3). The Borgloon soil has a finer texture and a larger organic carbon content than the latter soil.

The elucidation of microorganisms–substrates–soil structure interrelations is an important key for an improved understanding of organic carbon cycling in soils. Drying and rewetting of soils may be a useful tool for studying such relations. Determination of the effects of a disturbance of existing consumer–

substrates relationships will give more information on the situation before the disturbance.

CONCLUSIONS

The metabolic activity of microorganisms influences their survival during drying and rewetting of soils.

The enhanced mineralization after drying and rewetting of soils is partially derived from non-biomass soil organic matter, and partially from microbial biomass killed by drying and rewetting. The contribution of the non-living soil organic matter is determined by relations between soil microorganisms, organic components and soil structure.

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Effects of burrowing by the earthworm *Aporrectodea caliginosa* (Savigny) on beech litter decomposition in an agricultural and in a forest soil

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ABSTRACT

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A microcosm experiment was designed to study the effect of the endogeic earthworm *Aporrectodea caliginosa* (Savigny) on the C mineralization of ^{14}C -labelled litter in the upper 10 cm of an agricultural and of a beech forest soil. The following treatments were set up for both soils: (1) control; (2) +1 adult *A. caliginosa*; (3) daily mixing of the soils by hand; (4) as treatment (3) + addition of mineral N. The burrowing activity of *A. caliginosa* strongly stimulated ^{14}C mineralization. Mixing of soil and litter increased ^{14}C mineralization in the forest but not in the agricultural soil. The combined effect of mixing and N addition further increased ^{14}C mineralization in the forest soil and strongly increased it in the agricultural soil. While ^{14}C mineralization was still lower in treatment 4 than in treatment 2 of the beech forest soil, it was identical in those of the agricultural soil. It may thus be concluded that the effect of *A. caliginosa* on the mineralization of undecomposed litter in the agricultural soil is largely due to the combined effect of mixing of soil and litter and mobilization of N, while in the beech forest soil additional factors are important.

INTRODUCTION

In this paper, results from a laboratory experiment on the effect of the endogeic earthworm *Aporrectodea caliginosa* (Savigny) on leaf litter decomposition in an agricultural and in a beech forest soil, both developed from the same parent material, are reported. Changes in soil structure by bioturbation and related effects on nutrient availability are assumed to be the most impor-

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tant factors for earthworm induced alterations of decomposition rates (Lee, 1985). However, due to the cryptic life of soil biota and to the overwhelming importance of indirect relationships in soil (Anderson, 1988a, b), it is generally impossible to relate changes in process rates to certain activities or features of edaphic invertebrates (Wolters, 1991). Additional treatments were thus set up in which mixing of soil and litter as well as increased N availability were simulated by experimental manipulation. The comparison between these treatments and the *A. caliginosa* treatments allows a quantitative evaluation of the basic factors responsible for earthworm effects on decomposition rates. This experiment was intended to be an analytical approach to the effect of earthworms on microbial C use. It should be kept in mind, however, that this approach does not allow unequivocal conclusions on causal mechanisms.

MATERIALS AND METHODS

The sites where the soils were collected are situated in the Drakenberg area (agricultural soil) and in the Treppenweg area (beech forest soil) near Göttingen (Lower Saxony, Germany). Both soils are Rendzinas [Orthic Rendzina (FAO), Typic Rendoll (USDA)] and have developed from lower shell-lime (wave-lime) on a plateau about 350 m (Drakenberg) or 420 m (Treppenweg) above sea level. The site at the Drakenberg area has been under agricultural use with various crops for over 100 years, while the Treppenweg area is covered by 120–140 year old beech trees (Thöle and Meyer, 1979). Climate and soil conditions at the two sites are listed in Table 1. Total C and N were measured after dry combustion of air-dried material by a Carlo Erba ANA 1400 element analyzer.

The organic layers were carefully removed and soil from the upper 10 cm was collected four weeks before the experiment started. The soil was sieved (<4 mm), adjusted to 50% water holding capacity and stored at 4°C until use. At the beginning of the experiment, soil corresponding to 70 g dry weight

TABLE 1
Climate and soil conditions

	Beech forest soil	Agricultural soil
Rainfall (mm yr ⁻¹)	720	691
Mean annual temperature (°C)	7.9	7.1
Max. water holding capacity (% dry wt)	123.3	56.1
pH (H ₂ O)	8.3	7.9
Organic C (% dry wt)	8.31	3.25
Total N (% dry wt)	0.64	0.35
Organic C/total N	12.98	9.29

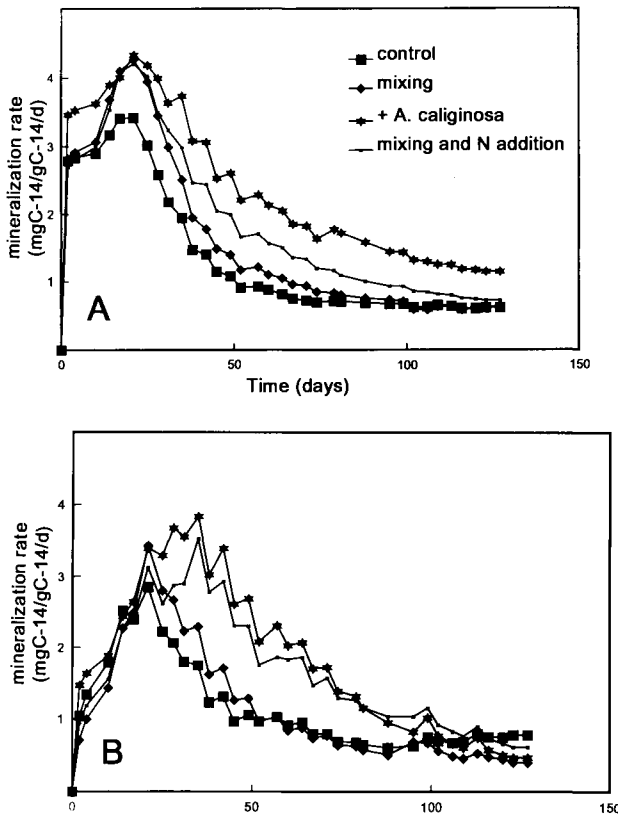


Fig. 1. Time-course of leaf-litter mineralization. (A) Beech forest soil. (B) Agricultural soil.

was filled into 20 one-liter Erlenmeyer flasks. Two days later (day 0), 68 mg of ^{14}C -labelled beech leaf litter were added to each flask to study the mineralization of undecomposed litter. The specific activity of the litter was $1 \text{ Bq } \mu\text{g}^{-1} \text{ C}$. Labelling and treatment of the litter have been described by Wolters (1989a). In short: the litter was broken down into small particles by means of an Ultraturrax. The soluble carbohydrates (corresponding to approx. 10% of the total carbon) were removed by extraction with a methanol-chloroform-water mixture to leave a residue of leaf fragments. Only the remaining leaf particles, referred to as "structural components of beech leaf litter" later in the text, were used for the experiment. The following treatments were set up with 5 replicates per treatment for both soils:

- (1) control;
- (2) + 1 adult *A. caliginosa*;
- (3) daily mixing of soil and litter with a spoon;
- (4) as treatment 3, + addition of $6.17 \text{ mg } (\text{NH}_4)_2\text{SO}_4$.

The amount of N supplied to treatment 4 equals the additional amount of

N mineralized during one day in the *A. caliginosa* treatment of the beech forest soil as determined in a pilot experiment. The experimental containers were stoppered with a rubber bung and incubated at 10°C under permanent darkness. They were opened daily for approximately 20 min to allow gas exchange. CO₂ evolved during incubation was absorbed in 1M NaOH and ¹⁴CO₂-C was measured in an aliquot by liquid scintillation counting (see Fig. 1 for time schedule of determinations). The experiment ran over 127 days. The significance of treatment effects on cumulative ¹⁴CO₂-C liberation was tested by means of a two-way ANOVA (soil × experimental manipulation). The difference between means was tested by the Tukey Test at the 5% level.

RESULTS

General effect of treatments

The results of the two-way ANOVA are summarized in Table 2. The mineralization of ¹⁴C-labelled beech litter in the forest soil was significantly different from that in the agricultural soil. Addition of *A. caliginosa* and other experimental manipulations significantly altered ¹⁴C mineralization. Significant interactions reflect the strong interrelationships between soil conditions and the effect of experimental manipulations. The LSD_{0.05} value revealed by the Tukey Test was 18.1 mg ¹⁴C g⁻¹ leaf litter C (Fig. 2).

Forest soil

A total amount of 159.3 mg ¹⁴C g⁻¹ leaf litter C was mineralized in the controls of the beech forest soil during the course of the experiment (Fig. 2). *A. caliginosa* stimulated the mineralization of the labelled leaf litter from the very beginning (Fig. 1A) and increased cumulative ¹⁴CO₂ liberation by 77% to 281.6 mg ¹⁴C g⁻¹ C (*P* < 0.05). Mixing of soil and litter significantly stimulated the C mineralization of the labelled beech leaf litter and this effect was further increased by mineral N addition. However, even in the latter treat-

TABLE 2

Results of the ANOVA

	SS (%)	F ^a
Soil (1) ^b	9.35	4.6*
Treatment (3)	53.64	39.2****
S×T (3)	16.82	9.34***
Error	20.23	—

^aSignificance of *F*-values; *, *P* < 0.05, **, *P* < 0.01, ***, *P* < 0.001, ****, *P* < 0.0001.

^bDegrees of freedom.

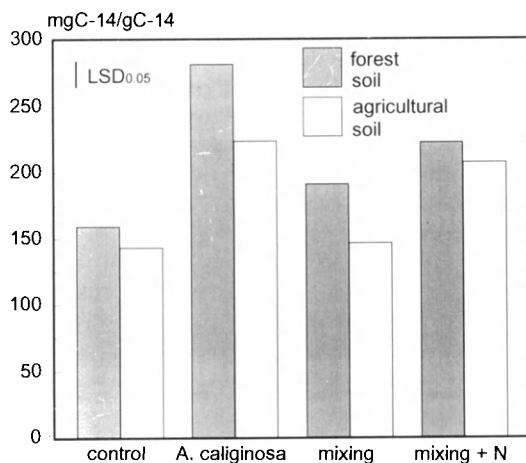


Fig. 2. Cumulative $^{14}\text{CO}_2\text{-C}$ liberation during 127 days.

ment, this stimulation was significantly lower than in the *A. caliginosa* treatment.

Agricultural soil

Cumulative $^{14}\text{CO}_2\text{-C}$ liberation was lower in all treatments of the agricultural soil than in the corresponding treatments of the beech forest soil (Fig. 2). *A. caliginosa* effects on $^{14}\text{CO}_2$ liberation set in later than in the beech forest soil (Fig. 1B). *A. caliginosa* raised the amount of C mineralized during the experiment by 56% from 143.3 to 224.0 mg $^{14}\text{C g}^{-1}$ leaf litter C (Fig. 2; $P < 0.05$). Mixing of soil and litter had no significant effect on cumulative C mineralization of the labelled beech leaf litter. In contrast, the combined effect of mixing and mineral N addition led to a strong increase in $^{14}\text{CO}_2\text{-C}$ liberation, which was not statistically different from the effect of *A. caliginosa*.

DISCUSSION

The burrowing activity of the endogeic earthworm *A. caliginosa* strongly enhanced the mineralization of leaf litter. This points to the most important effect of earthworm activity on microbial C use (Edwards and Lofty, 1977; Wolters and Joergensen, 1992). Comparing the effects of *A. caliginosa* in the agricultural soil with the effects of experimental manipulations, it may be concluded that the stimulation of C mineralization in the earthworm treatment of the agricultural soil could sufficiently be explained by a combined effect of bioturbation and N mobilization. The strong stimulative effect of N addition suggests that the limiting effect of N availability may be so strong in the agricultural soil that the litter colonizing microflora could not respond to

mixing of soil and litter until additional N was supplied. This may partly be associated with the low nitrogen status of this soil (cf. Table 1). In the beech forest soil, other factors, such as the strong limiting effect of P, may be of additional importance (Wolters, 1985; Scheu, 1990).

The data suggest that the short-term effects of *A. caliginosa* on C mineralization are due to mixing of soil and litter as well as to an alteration of nutrient availability and that these effects in turn are significantly affected by soil conditions. In addition, the data are consistent with the hypothesis that, in the Rendzina soil investigated, long-term changes in soil conditions as a result of agricultural practices reduce the ability of the soil microflora to decompose recalcitrant C compounds, such as structural components of beech leaf litter.

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The effect of location in soil on protozoal grazing of a genetically modified bacterial inoculum

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ABSTRACT

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Short-term laboratory experiments were performed to investigate the effect of location on protozoan grazing of a genetically modified bacterial inoculum in soil. *Pseudomonas fluorescens* (strain 10586, containing chromosomally borne genes encoding bioluminescence and antibiotic resistances) was introduced into varying pore size classes by adjustment of the soil matric potential with reference to the moisture release characteristic. The soil ciliate protozoan *Colpoda steinii* was subsequently introduced to the soil at conditions close to field capacity to ensure initial location in larger pores.

When the *Ps. fluorescens* was predominantly located in small pores (less than 6 μm pore neck diameter), the decline in viable cell concentration was less than that when located in larger pores. This suggests that the bacterial inocula introduced into soil may be protected by spatial compartmentalisation. This protection may be from protozoan grazing, in which case the predator activity of the introduced *Colpoda* inoculum was not significant in comparison to that of the indigenous protozoa. Further work is therefore required to determine the mechanism of protection but the findings demonstrate that the antecedent matric potential and pore size characteristics will be critical in determining the survival characteristics of microbial inocula in soil.

INTRODUCTION

The introduction of bacterial populations into the soil environment is frequently followed by a decline in viable cell concentration. This decline ceases when a characteristic survival concentration is reached, which appears to be independent of the initial inoculum size (Crozat et al., 1987). The grazing

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activities of indigenous protozoa are considered to be a significant component in the survival and establishment of bacterial inocula (Habte and Alexander, 1978).

The predatory activities of protozoa are considered to be affected by the matric potential of the soil, since protozoa are dependent upon water for their dispersal and movement through the soil (Sleigh, 1973). The largest protozoan populations are found to exist in saturated soils, and the lowest concentrations in dry soils (Darbyshire, 1976). Postma et al. (1989) observed an increased survival rate of bacterial cells introduced into relatively dry soils in comparison to those inoculated into wetter soils, and suggested that this resulted from reduced protozoan predation.

The heterogeneous and discontinuous structure of soil provides a number of distinct or temporally discrete microhabitats, which are influenced by environmental fluctuations (Hattori and Hattori, 1976). Alexander (1981) proposed the importance of such microsites in the survival of bacterial inocula where they exclude the predator and protect the prey.

At low matric potentials soil water will be restricted to those pores with small size diameters. The predatory activities of the protozoa under such conditions are thought to be restricted, since they are denied access to their prey due to their larger size. Postma and Van Veen (1990) described the habitable and protected pore space in the soil matrix by varying the matric potential of the soil with reference to the moisture release characteristic. Heijnen and Van Veen (1991) found that pores with neck size diameters less than $6\text{ }\mu\text{m}$ positively affected the survival of introduced bacteria, whereas pores with neck size diameters greater than $6\text{ }\mu\text{m}$ had a negative effect.

This article describes short-term laboratory experiments performed to investigate the effect of location on protozoal grazing of a genetically modified bacterial inoculum in soil. Bacterial inocula were located in distinct pore size classes with reference to the soil moisture release characteristic. Protozoa were introduced at matric potentials sufficient to ensure their location in larger pores. Introduction of bacteria into pores with small neck size diameters ($<6\text{ }\mu\text{m}$) was intended to spatially compartmentalise the predator and prey.

MATERIALS AND METHODS

Soil type and preparation

A sandy loam soil from the Insch series (Grid ref. NJ659223) was used. Soil samples were taken from grass ley sites ensuring minimal pesticide contamination. The soil was sieved to collect the fraction of particle size less than 3 mm and was air dried and stored at 4°C . The soil moisture release characteristic was determined by equilibration of initially saturated soil on pressure membrane apparatus. Figure 1 shows the relationship between soil matric

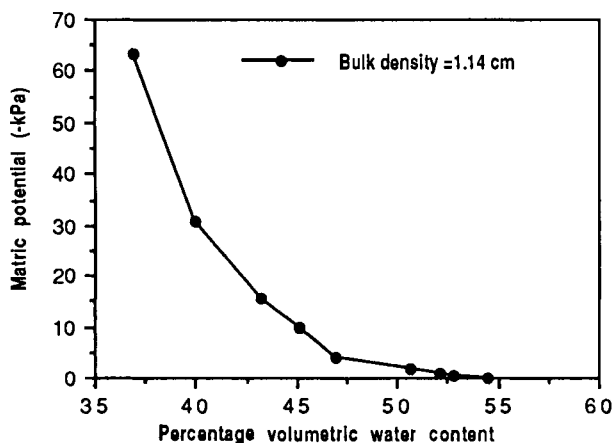


Fig. 1. Moisture release characteristic of repacked Insch soil.

potential and moisture content of repacked Insch soil with a bulk density of 1.14 cm^{-3} .

Bacterial strain and growth conditions

Pseudomonas fluorescens 10586s FAC510 was kindly donated by S. Amin-Hanjani and contains chromosomally borne *lux A* and *B* genes and genes encoding resistance to kanamycin, spectinomycin, ampicillin and rifampicin. Incorporation of these genes has no detectable effects on the specific growth rate or fitness of the host strain (S. Amin-Hanjani, pers commun., 1991). The bacterium was routinely cultured on LB-broth containing $20 \mu\text{g}$ kanamycin ml^{-1} at 30°C on a rotary shaker (150 rpm) for 48 h. Prior to inoculation, cells were grown to the mid-exponential phase and harvested by centrifugation ($8800g$). The cells were resuspended in sterile phosphate buffer (15 mM) and starved overnight at room temperature.

Protozoan strain and growth conditions

Colpoda steinii, an indigenous soil protozoan, was kindly donated by Dr. J. Darbyshire. The protozoa were cultured on *Pseudomonas fluorescens* cells in Stanier's medium (Stanier, 1947) supplemented with 0.1% peptone and 0.1% glucose.

Soil microcosm studies

Short-term studies of survival of *Pseudomonas fluorescens* were carried out in soil microcosms, consisting of 50 mm (diameter) sterile Petri dishes into which 9.59 g air-dried soil was placed. The soil formed a 6 mm deep layer

corresponding to the height of two soil aggregates. Microcosms were maintained in polystyrene boxes at 25°C in a constant temperature room for a period of 270 h.

Two treatments were involved in the short-term experiments. In Treatment 1 the bacterial inoculum was located in pores with neck size diameters $< 6 \mu\text{m}$, whilst a buffer zone created by the addition of water separated the bacteria from the protozoa, which were situated in pores with neck size diameters between 30 and 60 μm . In Treatment 2 the small pores ($< 6 \mu\text{m}$) were filled by water. The bacterial inoculum was located in pores with neck diameters 6–30 μm adjacent to the protozoan inoculum, which was located as in Treatment 1 (Fig. 2).

The soil matric potential was raised to -5 kPa over a 30 h period. The bacterial and protozoan inocula, along with the water to create buffer zones, were evenly distributed over the soil surface in a dropwise manner. Even distribution was facilitated by placing a 1 cm^2 grid over the microcosm surface during inoculation and allowing sufficient time for equilibration.

The matric potentials necessary to locate inocula in distinct pore size classes ($< 6 \mu\text{m}$, 6–30 μm and 30–60 μm) were determined by calculating the effective pore neck size diameter (d , μm) as $d = 300/\text{matric potential in kPa}$. The volumetric water contents required to achieve the desired soil matric potentials were derived with reference to the moisture release characteristic (Fig. 1). The initial matric potential in the microcosms (-50 kPa) was confirmed using the filter paper method described by Graecen et al. (1989), after a 24 h

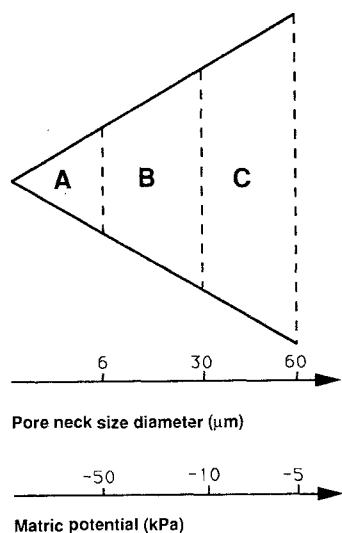


Fig. 2. Schematic representation of the pore neck diameters in which the inocula were located and the matric potential of the soil after equilibration. Treatment 1: A=bacteria, B=water, C=protozoa/water. Treatment 2: A=water, B=bacteria, C=protozoa/water.

equilibration period. The matric potential was raised to -10 kPa over a 6 h period and finally to -5 kPa at 30 h.

Sampling procedure

Triplicate microcosms were sampled destructively at 0, 24, 30, 54, 102, 150, and 270 h. Soil samples (0.5 g) were transferred to 1 ml phosphate buffer (15 mM) and vortexed for a 10 s period. Viable cell concentrations of *Pseudomonas fluorescens* were determined by dilution plating on *Pseudomonas* minimal medium (Panopoulos et al., 1975) containing 20 μg kanamycin ml^{-1} , 50 μg ampicillin ml^{-1} , 50 μg spectinomycin ml^{-1} and 100 μg cycloheximide ml^{-1} . The effect of location and the presence of protozoa on the bacterial inoculum population dynamics was examined with two way analysis of variance using the Minitab statistics package.

RESULTS

The influence of the location of *Pseudomonas fluorescens* in pores with small neck size diameters ($< 6 \mu\text{m}$) in the presence and absence of a *Colpoda steinii* inoculum (final concentration 5.4×10^2 cells per g oven-dried soil) was studied in Treatment 1. At 0 h the bacterial inoculum (final concentration 8.1×10^7 viable cells per g oven-dried soil) was added to the microcosm. The filter paper method confirmed the location of the liquid component of the inoculum in pores with neck sizes $< 6 \mu\text{m}$, since the water content of the filter paper corresponded to a suction of -54 kPa. It has shown that different distribution patterns of bacterial cells can be achieved by inoculating at different initial moisture contents (Postma et al., 1989). However, adsorption of bacterial cells to soil sites other than those desired may have occurred particularly at low matric potentials. Viable cell concentrations in the sample taken at 0 h were less than those inoculated, due to difficulties in extracting all cells from soil and possible cell death resulting from the different environmental conditions. In the subsequent 24 h the viable cell concentration rose to 1.1×10^8 cells per g oven-dried soil. The increase in matric potential to -5 kPa at 30 h was followed by a decline in viable cell concentration by approximately two orders of magnitude over a 240 h period in the presence and absence of *C. steinii* (Fig. 3a). The survival rate of *Ps. fluorescens* in pores with small neck size diameters was not significantly different in the presence and absence of *C. steinii* (5% level of significance).

In Treatment 2 the decline of *Ps. fluorescens* viable cell concentration located in larger pores ($6\text{--}30 \mu\text{m}$) was studied in the presence and absence of a *C. steinii* inoculum (final concentration 5.4×10^2 cells per g oven-dried soil). At 24 h the bacterial inoculum (final concentration 7.2×10^7 viable cells per g oven-dried soil) was introduced to the microcosm to raise the matric poten-

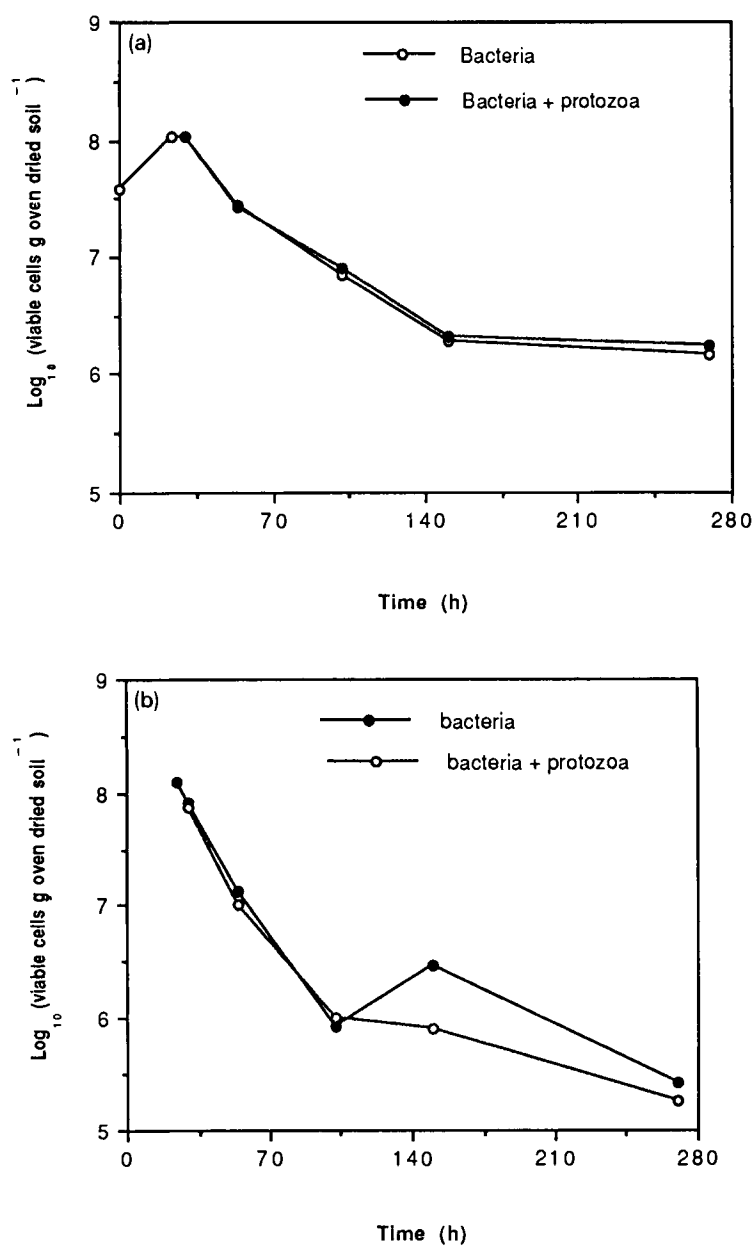


Fig. 3. (a) Population dynamics of *Pseudomonas fluorescens* located in pores with neck diameters less than $6 \mu\text{m}$, in the presence and absence of *Colpoda steinii*. The majority of standard error values were less than 15%. (b) Population dynamics of *Pseudomonas fluorescens* located in pores with neck diameters between 6 and $30 \mu\text{m}$, in the presence and absence of *Colpoda steinii*. The majority of standard error values were less than 15%.

tial to -10 kPa. The viable cell concentration declined by approximately three orders of magnitude in the presence and absence of *C. steinii* (Fig. 3b). The population dynamics of *Ps. fluorescens* located in larger pores did not differ significantly (5% level of significance) in the presence and absence of *C. steinii*.

The apparently different survival rates of *Ps. fluorescens* located in distinct pore size classes were analysed. It was found that the survival of bacteria located in pores with small neck size diameters was significantly higher, in the presence (5% level of significance) and absence (1% level of significance) of protozoa, than when located in pores with larger neck size diameters.

DISCUSSION

Pseudomonas fluorescens located in pores with neck size diameters $< 6 \mu\text{m}$ exhibited greater survival than those situated in pores with neck size diameters between 6 and $30 \mu\text{m}$. The addition of *Colpoda steinii* produced no significant effect on the bacterial populations located in the two pore size classes. This lack of influence may be due to the presence of concentrations of indigenous soil protozoa at levels equivalent to that provided by the inoculum, or with greater activities. Sleigh (1973) reported that protozoan numbers in moist soil may range between 10^3 and 10^5 cells per g soil. Postma and Van Veen (1990) found a more pronounced decrease in rhizobial cells in natural soil than in sterile soil at higher soil moisture contents. This decrease was attributed to biotic factors such as protozoan predation. The protozoan inocula provided 5.4×10^2 cells per g soil, which may not have significantly increased predation above that carried out by the indigenous population.

Previous attempts to describe the effects of bacterial location in distinct pore size classes on inoculum survival have relied on the introduction of inocula into soils of varying moisture contents (Postma and Van Veen, 1990; Heijnen and Van Veen, 1991). The observed increase in survival of introduced rhizobia in drier clay treated soils compared to wetter ones may be attributable to a discontinuous water film present in drier soils (Heijnen and Van Veen, 1991). Vargas and Hattori (1986) demonstrated the reduced grazing activity of protozoa in dry soils. This may be considered in terms of their exclusion from pores containing their prey due to their size, or their dependence on sufficient soil water for dispersal and movement. Similar studies were carried out by Kuikman et al. (1990), although in their experiments the final matric potential was much lower than used here, restricting the movement of protozoa. In addition, protozoa and bacteria were inoculated into different portions of soil before mixing. The experiments discussed in this paper were performed at close to field capacity (-5 kPa), thereby eliminating any reduction in protozoal grazing activity due to insufficient soil moisture which may restrict the movement or dispersal of protozoa. The enhanced

survival of bacteria located in pores with small neck diameters is therefore thought to be due to the pore size characteristics rather than the moisture content of the soil. This suggests that the antecedent matric potential and the pore size characteristics will be critical in determining the survival of microbial inocula, genetically modified or otherwise, in soil.

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The relevance of soil physical measurements on the macroscale for the description of biological processes on the microscale — Introduction to round table discussion

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SUMMARY

The scale of physical measurement in relation to the activity of soil organisms depends on the objectives of the study.

It is suggested that while it is always important to explore detail at all levels of scale, most practical problems relating to the physiology of the organisms and to their effects as populations in the field, are most appropriately studied at a macroscopic level using macroscopic physical measurements with all the conceptual difficulty that this approach entails. Conventional technology is available for these measurements, but there is a clear need for closer collaboration between biologists and soil physicists to ensure that full advantage is taken of complementary and focussed skills.

INTRODUCTION

Productive and sustainable management of land for both agricultural and non-agricultural purposes demands knowledge of the soil environment and its consequences for soil biota. The discussion which follows considers the need to define physical aspects of that environment and to identify scale of measurement most appropriate to that definition. In particular, we examine the relevance of macroscopic physical measurement to microscale biological processes.

To further focus the discussion we shall concentrate on organisms in the size range $10^{-7} < L/m < 10^{-4}$, where L is a characteristic dimension of the organism. This range includes bacteria, fungal hyphae, nematodes and root hairs. The upper end of the range corresponds to soil pores which drain at suctions of approximately 10 kPa.

In choosing this range, we avoid consideration of the effects of soil strength but recognise the possible importance of water availability and osmotic pressure, effects of gas movement and thermal effects. We also note that the mobility of many of the creatures in this size range depends on water content and water movement.

ILLUSTRATIVE ISSUES

To further focus discussion, it is useful to examine objectives of study of the soil microbiota. There appear to be at least three important, distinct but interrelated problems:

- (1) The basic physiology of soil organisms and its response to the local physical environment.
- (2) The field behaviour of populations of organisms and the way this behaviour is affected by management.
- (3) The effect of scale and soil heterogeneity in relation to these problems.

The first of these problems reveals biological opportunity as well as the measurements necessary to adequately qualify field behaviour so that it can be generalised in space and time. The second and third, in principle, provide the information to manage, for example, elements of the nitrogen and carbon cycles, pathogenicity associated with soil organisms and its biological control, and the movement of microbiological populations in soils.

The nature and complexity of these problems are different. Basic physiological questions of particular species, in principle, permit relatively simple resolution because gaseous, thermal and liquid environments can be controlled with precision and the biological effects measured accurately, often *in vitro*. Griffin (1972) and more recently Yates and Yates (1988) explore many of these issues. It must be observed, however, that many such studies described in the literature appear complicated by confusion of objectives and premature recourse to the complexities of the field environment.

The field problem is much more complicated because of effects of competition among and between species of organisms. In fact, it is unlikely that we could ever integrate individual activities to derive a quantitatively correct population response to some arbitrary change in even one physical property of the environment. This problem appears to lie in the realm of "trans-science" (Weinberg, 1972) in which scientific (and social) decisions and predictions are made on the basis of incomplete data, uncertain theory and (generally conservative) "judgment". Weinberg nevertheless asserted that methods of science still serve to "inject intellectual discipline into the republic of trans-science".

Field application is also complicated by the scale and heterogeneity of soil properties and the need to "match" soil measurement to the scale of the problem. Philip (1980) referred to, and extended, the notions of Weinberg to this domain in a survey of many problems associated with field application of soil physics theory.

Having made this point, it must be admitted that scaling theory such as that of Miller and Miller (1956, 1990), and approaches based in fractal geometry (e.g., Tyler and Wheatcraft, 1990) offer opportunities to transcend scale in

some classes of porous material structure. These approaches offer no solution, however, to the biological population and competition problem.

It remains important, however, even if elements of the problem defeat deterministic solution, to retain appropriate physical definition of the environment where possible in order to retain some rigour in the sense sought by Weinberg.

We therefore examine basic elements of macroscopic soil physics theory to see what it offers the soil microbiologist.

MACROSCOPIC SOIL PHYSICS THEORY

Local variation in soil water, heat and gas flow at the pore and aggregate scale in soil is great. It is, nevertheless, possible to identify a "representative elementary volume" (Youngs, 1964) containing many pores and particles within which microvariation is smoothed out and which provides a basis for a continuum (Raats and Klute, 1968) approach to heat, water and gas flow at what, for water, is called the Darcy scale (Philip, 1970).

Within this scale, we formulate a continuity equation which relates the rate of change of the amount of the entity in an arbitrary volume element, to its net flux into that element. In addition, we must relate the flux of the entity to a space gradient of its "potential".

This approach pre-supposes an ability to measure reliably and repeatedly the constituent variables of the theory. In the case of soil water, for example, the approach requires unambiguous definition of the water content and the hydraulic conductivity, as functions of the water potential. The conductivity is a measurable, but not predictable, proportionality relating water flux to the soil water potential gradient.

The water content and conductivity functions of soil are necessary and sufficient to fully describe and predict water flow in that soil in response to appropriate sets of initial and boundary conditions. The theory finds its authority in experimental tests, and is well set out in soil physical texts such as Baver et al. (1972), Hillel (1980), Marshall and Holmes (1985). Analogous approaches for salt, heat and gas flow are also set out in these texts.

When one seeks to describe the physical environment of soil organisms, one is confronted with an essentially similar spatial and variability problem so individual microorganisms experience conditions within representative volume elements which differ markedly from the element average.

Four years ago, I explored these issues (Smiles, 1988) and suggested that physical conditions affecting soil organisms should be considered at a scale of the organism. I also pointed out the need to discriminate between *quantities* of entities such as water and their corresponding *potentials*. I suggested that except for cases of local disequilibrium (often generated by the organisms), it seemed that the potential of an entity transcends scale, so macroscopic mea-

surements of water potential, temperature or gas partial pressure in the soil appropriately specify that aspect of the environment for all scales. On the other hand, the volume fraction of the water as a measure of soil water content reveals little of the local distribution of the soil water, except that which might be inferred from the soil moisture retention function using quite simplistic soil pore models (Smiles, 1988).

When these arguments are revisited in relation to the illustrative objectives of study identified above, however, they appear too simplistic. So while we can often describe (using precise micro-histological and chemical methods) biochemical consequences in individuals following changes in their immediate environment using micro-histological and chemical methods, the most reliable measurement of activity of field importance, such as organic matter breakdown, methane production, or pathogenicity, must be based in integrated effects of the population of distributed and variously active individuals.

It thus appears that microbial consequence of soil physical change will be best quantified only within a volume analogous to the representative volume of physical discourse. Macroscopic soil physics theory at this scale then seems to provide an entirely appropriate basis for describing it.

This also seems true in relation to movement of organisms with, and relative to, water in soil. Tan et al. (1991), for example, describe very well the movement of *Pseudomonas* with, and relative to, water during unsteady, unsaturated absorption of water by soil using a macroscopic approach. They also identify effects attributable to soil solution salt concentration which arise because many microorganisms develop reversible physico-chemical associations with soil colloids (Roper and Marshall, 1978), and which may protect as well as effectively immobilise the organism. In this context, Heijnen and van Veen (1991) review evidence for protection of bacteria against predation by amoebae that are not able to enter small ($< 6 \mu\text{m}$) pores in bentonite-amended sandy soil.

Of course, it remains necessary and appropriate to define detail of processes in identifiable structural elements at a scale less than the representative elementary volume where this a concern. The paper by Rappoldt (1990) summarised in this symposium, and that of Leffelaar (1991), which examine the application of macroscopic diffusion theory to aggregated soils are cases in point. The use of statistical theory to describe and relate penetrometer response during short range insertion to microbiological attributes (E. Perfect, pers. commun., 1991), is another example. Generally, these intuitions must appeal to macroscopic measurement, however, for ultimate test.

Finally, it is important to recognise that a microorganism is a discriminating sensor and the effect of physical attributes of its environment must be interpreted with care. Soil water potential, for example, has a matric component which arises from the interaction of water with the soil surfaces and their geometry. There is also an osmotic component. A nematode, for example,

which is wholly immersed in water but does not distort an air–water interface, is relatively insensitive to the matric component of the soil water potential, although it must respond to the osmotic potential in proportion to the osmotic selectivity of its membranes. On the other hand, the mobility of the organism must be wholly constrained by the water films about and within soil particles, and is therefore strongly water content dependent.

With these qualifications, however, soil microbiological function appears to be systematically measurable only in an average sense, at a macroscopic level, and therefore an appropriate subject of macroscopic theory.

In conclusion, it is important to note that for too long there has been inadequate dialogue between soil scientists and chemists and soil biologists. Physicists have not therefore been sufficiently aware of opportunity and need at this level, and existing methods well described, for example, in Klute (1986) are not as well tailored to biological need as they might be. These deficiencies must be addressed however if soil biological experience, particularly in the field, is to be raised to a level that is more than anecdotal and becomes formally extendable in space and time within a secure framework that can match measurable environmental conditions to microbiological response.

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SUMMARY OF ROUND TABLE

Crawford, UK: Doesn't agree that the macroscopic scale is always relevant and/or provide insight into the causal relationships between environmental conditions and their effects on micro-organisms. The dynamics of a disease across the UK at the national level was stochastic and random, but at a more detailed scale of cities the dynamics of the disease could be understood in terms of non-linear causal relationships. The same may be true for soil processes.

Rappoldt, The Netherlands: Agrees with Crawford. For example, diffusion processes associated with biological activity take place and can be measured and understood only at a mm scale. But it is uncertain whether models for these diffusion processes can be scaled up to the field level.

Crawford, UK: The representative elementary volumes provide some problems. They are certainly different for roots and for microbes. How are you going to handle the interaction between the two in that case.

Bouma, The Netherlands: Representative elementary volume (REV) is focussed on the soil aspect. REV is useful, but within the REV there are microprocesses going on. We should realize that the REV may act quite differently depending on the boundary conditions.

Raats, The Netherlands: Agrees that scaling up is a difficult problem. The example given by the Chairman about scaling up water movement is really quite simple compared to scaling up some other processes, e.g., gas diffusion. Scaling up from an intermediate (macro) scale to the field scale is done often, e.g., with nutrient and pollutant movement, by building in experience about the macroscale in models describing the field scale. Population dynamics has gone beyond considering populations as homogeneous: better theories now include size and age structure, and there is a large body of theory about such structured populations, which may be worthwhile pursuing by soil biologists. Soil biologists could try to set up models involving classes of soil biota and their interrelationships and transfers, analogous to mobile and immobile phases in soil physical models.

Tomlin, Canada: In spite of great difficulties, we do scale up from reactions of individual plants to global scale. Why couldn't we do the same from microbes to the whole soil scale?

Kuikman, The Netherlands: Gene transfer among microbes takes place at the microscale, but no model is available to describe/explain that process. The soil physicists have to come down to the microscale and explain well known phenomena to them (e.g., hysteresis) that oppose (?) a stop and start mechanism to microbes. Such processes would inhibit easy upscaling.

Raats, The Netherlands: The fascination of soil physicists with the macroscale is quite understandable, but there is indeed need of development of techniques at the small scale by soil physicists. Examples: micro-O₂ electrodes, mini-tensiometers. Further development of such techniques deserves more attention.

Priesack, Germany: Waterflow at the microscale is difficult to describe: e.g., moving boundaries of sections between air and water cannot yet be described properly, which is one of the main problems we have to tackle at the microscale.

Raats, The Netherlands: Soil physicists are doing okay at the intermediate scale but are generally doing poorly at smaller and larger scales. A favorable and reasonably successful exception is using macroscopic measurements on water retention to infer information about pore size distribution.

Rappoldt, The Netherlands: Adds it may be useful to distinguish between prediction and description when discussing these matters.

Bouma, The Netherlands: Two points: (1) Scaling up from REV's to horizons to profiles to landscape is a procedure that many people are working on. The same procedure would seem to be logical when going up from microscale. (2) The very strong deterministic attitude of soil biologists is quite striking, but a question is whether we should really focus on stochastic approaches.

Koehler, Germany: In addition to external effects, we should consider inherent properties of soil organisms in trying to understand their reaction to external stimuli. Considering only external conditions, even in all possible detail, therefore does not always yield interpretable results.

Chairman: I propose we might consider a minimum set of measurements to characterize the environment; would anyone like to comment on, or add to: O₂, salt concentration, suction, bearing in mind that matric potential may be less important for immersed organisms.

Winter, Canada: O₂ is only relevant at the microscale, not at the macroscale. For denitrification you need many micromasurements!

Filser, Germany: One should pay most attention, however, to water content, bulk density and carbon and nitrogen content. These will give us at least an idea of the potential biomass. There is hardly a factor that does not influence soil fauna. Maybe limnological approaches can be used in the sense that (soil) organisms can say something about the state of the soils, just as aquatic biota inform us about the state of the water.

Chairman: What about water potential and temperature?

Ingham, USA: The abiotic factors mentioned are all important for defining the niche for micro-organisms: I would add nutrient resources to the list.

Filser, Germany: Temperature should be measured continuously: the peaks are more important than intermediate values. This may also hold for other parameters.

Chairman: Calls it a day and wishes everyone a good meal.

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Interrelationships between soil structure/texture, soil biota/soil organic matter and crop production

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ABSTRACT

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Interrelationships between soil structure/texture, soil biota/soil organic matter and crop productivity occur from a scale of micrometres to metres. In this paper an attempt has been made to integrate and quantify these interrelationships by using the themes of C and N mineralization and porosity using data from soils in western Canada. Although the concept of soil porosity (texture and structure) has been used to describe water and air movement in soil, it has not been fully explored to describe microbial, faunal and plant interactions. Future work has to incorporate the effects of soil porosity on biological processes in agro-ecosystems.

INTRODUCTION

Net primary production is an integrative measurement of numerous physical, chemical and biological processes operating within and outside the soil. In the field of soil microbiology and biochemistry, an understanding of the composition of soil microorganisms and their role in decomposition and nutrient cycling in agro-ecosystems has received a great deal of attention in the past 30 years. Measurement of the activity of organisms and their impact on various processes has led to a development of a variety of simulation models which have been used as tools of integrating existing knowledge and for testing hypotheses. In contrast, work in soil zoology and micromorphology has examined the role of fauna and plant roots in soil structure formation and alteration of porosity. Observations of soil thin section show clear evidence of plant root and faunal activity but quantification of soil porosity and its

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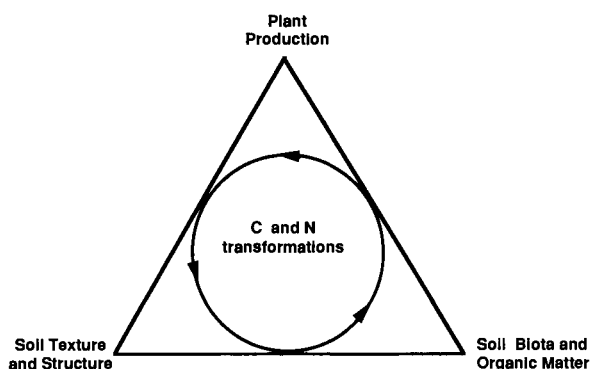


Fig. 1. A conceptual framework for studying the interrelationships of soil structure/texture, soil biota/soil organic matter and plant growth using the themes of porosity and mineralization of C and N. The impact of soil texture and structure on various processes occurs through changes in porosity.

impact on soil processes has been achieved to a limited extent. Porosity affects the microbial–faunal interactions, aeration and water relations, thus soil fauna and plants may have direct and indirect effects on a number of soil processes. There is a need to develop concepts to integrate information obtained at various scales of resolution and from various disciplines to understand controls on crop production. The objective of this work was to develop a framework to quantify the interrelationships between soil structure/texture, soil biota/soil organic matter and crop production by using porosity and mineralization of carbon and nitrogen as integrating themes (Fig. 1).

HOW TO ASSESS INTERRELATIONSHIPS BETWEEN SOIL STRUCTURE/TEXTURE AND SOIL MICROBIAL–FAUNAL ACTIVITY THROUGH CARBON AND NITROGEN TRANSFORMATIONS

In order to understand some of the interrelationships between soil structure and texture on microbial and faunal activity, it is necessary to quantify pore size distribution, microbial and faunal populations, and C and N dynamics. Although a number of microscopic and submicroscopic techniques are available to study the organisms, organic and inorganic components, and pore size distributions, a coarser-scale of resolution was chosen to integrate the information because it allowed the use of knowledge from other disciplines of soil science, especially soil physics.

Literature review of methods used

Texture and structure affect pore space, bacterial and faunal populations and their activity in soil. Pore size distribution may be determined by image

analysis or by camera lucida (Darbyshire et al., 1985, 1989). Effective pore size distribution may be estimated from the soil moisture retention curve by the capillary rise equation (Hattori and Hattori, 1976; Papendick and Campbell, 1981; Elliott et al., 1984). Papendick and Campbell (1981) showed that the proportion of pores equal or less than $3\text{ }\mu\text{m}$ were 72% for clay, 40% for silt and 22% for sandy soils. Fine-textured soils generally have a larger proportion of small pores than coarse-textured soils.

Habitable pore space accounts for a small portion of total pore space and affects bacterial and faunal distribution in soils. Microorganisms and small fauna require water films, hence most decomposition of organic matter occurs within water-filled pores (Bamforth, 1988). Bacteria probably have the most favorable environment in aggregates with small diameter pores. These pores retain water longer in a drying soil and may offer better protection from faunal predators than larger pores and aggregate surfaces (Hattori and Hattori, 1976; Vargas and Hattori, 1986; Foster, 1988). Up to 60% of soil bacteria pass through a $0.8\text{ }\mu\text{m}$ membrane filter (Bakken and Olsen, 1987). Since there is approximately a 3:1 ratio between the diameter of pores and the diameter of bacteria (Kilbertus, 1980), a large proportion of bacteria may occupy pores $<2.4\text{ }\mu\text{m}$ diameter. This small pore size may restrict predation of bacteria by some fauna, such as nematodes and large ciliates. However, some small protozoa can occupy pores down to $2\text{ }\mu\text{m}$ diameter (Bamforth, 1985), potentially allowing them to be effective predators of many bacteria. Bacteria contained within pores $<2\text{ }\mu\text{m}$ would be "protected" from protozoan predation. More research is needed to elucidate the effects of pore space on bacterial population size and grazer activity (Elliott et al., 1980).

In arable soils free-living protozoa affect the number of bacteria and influence nutrient cycling in the short term (Clarholm, 1984; Bamforth, 1985). Microcosm studies have shown that microbial turnover rates and mineralization often are increased when microbial predators are present (Anderson et al., 1981; Coleman et al., 1984; Clarholm, 1985; Griffiths, 1986; Kuikman and Van Veen, 1989). When fauna respire CO_2 —C, N should be mineralized because the C:N ratio of fauna are similar to the bacteria that are grazed (Coleman et al., 1984). Nitrogen mineralized by protozoa makes N more available to microbes and plants under N limiting conditions and may be important in decreasing the limitation on plant growth or microbial decomposition rate of high C/N ratio substrates (Anderson et al., 1981; Clarholm, 1985; Robinson et al., 1989).

Turnover of C and N within the microbial biomass is faster in coarse-textured soils than in fine-textured soils (Van Veen et al., 1984, 1985; Merckx et al., 1985). Textural effects may impose physical restrictions on the ability of fauna to graze on microbes, therefore texture may play a role in faunal-induced mineralization of microbial C and N. Elliott et al. (1980) found, in part, that amoeba grazed bacteria more in a sandy loam subsoil than in a clay

subsoil. This was characterized by higher amoebal numbers and a greater increase in $\text{CO}_2\text{-C}$ evolution in the sandy loam. The effect of texture on N mineralization was not reported. Gupta and Germida (1989), studying the protozoa-induced mineralization of bacterial sulfur, found that amoeba in a loamy sand soil reduced bacterial numbers more than in a sandy clay loam soil. The rate of $\text{CO}_2\text{-C}$ production in the loamy sand increased more than in the sandy clay loam when protozoa were added. They thought that a higher number of large pores in the loamy sand soil could explain the larger grazing effect compared to the sandy clay loam soil.

In order to generalize results, it is necessary to link the dynamics of microbial and faunal populations to soil processes (Richards, 1974). One approach is through simulation modelling. A variety of simulation models describing the mineralization and immobilization of N (Frissel and Van Veen, 1981; Juma and Paul, 1981; McGill et al., 1981; Voroney et al., 1981; Van Veen et al., 1984; Parton et al., 1988) and food webs (Hunt et al., 1984, 1987, 1989; Moore et al., 1990) already exist. Most soil organic N models emphasize the dynamics of microbial biomass. In contrast, soil food web models describe the microbial-faunal interactions explicitly but do not describe soil organic matter dynamics adequately. Few studies have reported the effects of soil texture on bacterial-protozoan interactions in soil, including C and N mineralization (Rutherford, 1991).

Effect of texture/structure on microbial and faunal interactions and C and N dynamics

An integrated study to measure the effects of texture on dynamics of bacteria, C and N mineralization in the presence and absence of predators, and quantify the effect of predators on the flux of C and N in soils with different textures was conducted by Rutherford and Juma (1992a-d). A summary of the approach and results obtained is presented below.

The microcosm experiment consisted of samples from three sterilized Typic Cryoborolls (SiC, CL and SL texture) (Table 1) inoculated with *Pseudomonas* bacteria, two treatments (with and without protozoa), and five sampling dates. The *Pseudomonas* population was labelled in situ by adding glucose- ^{14}C and $\text{KNO}_3\text{-}^{15}\text{N}$ (Day 0). A species of *Acanthamoeba* was added to the microcosms on Day 2. Detailed descriptions of the experimental design and procedures are given by Rutherford and Juma (1992a, b).

The initial effective pores between 0.75 and 2.0 μm effective diameter made up 4.3, 3.1 and 2.4% of the soil volume for Soil A, Soil B and Soil C, respectively. It was assumed that bacteria contained within this pore size range were protected from protozoa predation. Pores between 2.0 and 9.7 μm effective diameter made up 7.0, 5.6 and 3.4% of the soil volume for Soil A, Soil B and Soil C, respectively. It was assumed that protozoa would inhabit pores within

TABLE 1

Selected chemical and physical properties of the three Typic Cryoborolls

Soil	pH ^a	C (%)	N (%)	Sand (%)	Silt (%)	Clay (%)	Texture ^b	Bulk density (g cm ⁻³)
A	6.7	8.19	0.62	9	46	45	SiC	0.85
B	6.8	5.14	0.42	37	31	32	CL	0.95
C	6.5	2.60	0.31	74	9	17	SL	1.02

^a1:2 soil:water.^bParticle size analysis was done by the hydrometer method.

this range. The ratio of "protected" pore volume (0.75–2.0 μm effective diameter) to "non-protected" pore volume (2.0–9.7 μm effective diameter) was 1.6, 1.8 and 1.4 for Soil A, Soil B and Soil C, respectively.

On Day 4 bacterial numbers in all three soils were approximately 3×10^9 g⁻¹ soil. The greatest reduction of bacteria due to protozoan grazing occurred between Day 4 and Day 7 (Rutherford and Juma, 1992b). Compared to the protozoa-minus treatment, bacteria in the protozoa-plus treatment were reduced by 68, 50, and 75% in Soil A, Soil B and Soil C, respectively. On Day 4, two days after protozoa inoculation, protozoa were all active. Numbers were 10,330, 4760 and 15,380 g⁻¹ soil for Soil A, Soil B and Soil C, respectively. Between Day 4 and Day 7, the period of greatest bacterial decline, total protozoa increased greatly to 150,480, 96,160, and 192,100 g⁻¹ soil for the three soils, respectively. Most protozoa became encysted by Day 7. Our results support the hypothesis that bacteria are more protected from protozoan predation in fine-textured soil than in coarse-textured soil.

All soils showed increased significant CO₂-¹⁴C evolution and NH₄-¹⁵N mineralization due to protozoan grazing. The mineralization rate of labelled N in the Soil C was much greater than in Soils A and B. Protozoan induced effects were transient in the soils studied and were most apparent in the coarse-textured soil (Rutherford and Juma, 1992b).

Experiments described above led to the development of a new model to describe bacterial–protozoan interactions and their impact on C and N cycling in two soils with different textures. This model integrated the concepts of soil organic matter as described in the Tramin model (Juma and Paul, 1981) and the bacteria–protozoa component of the food web model (Hunt et al., 1984). The unique features of this model are: (i) it combines the food chain with specific soil C and N pools, and (ii) it simultaneously traces the flows of C, ¹⁴C, N and ¹⁵N. A simplified flow chart is presented in Fig. 2.

Linkage of physical characteristics to biological processes in soil

In order to link the physical characteristics of soils to the biological pro-

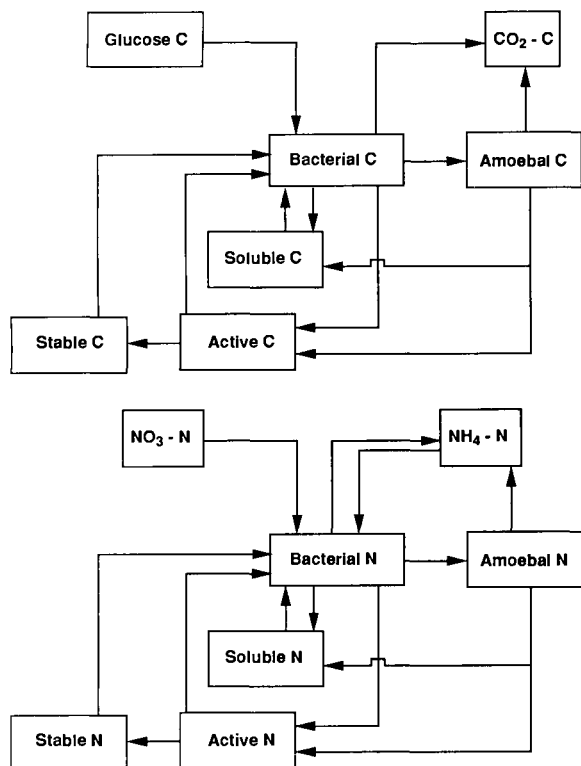


Fig. 2. Flow chart of C and N transfers in the simulation model describing bacterial amoebal interactions and organic matter dynamics in soil.

cesses in soil and to simulate the predation of bacteria by protozoa in a clay loam soil, we described the consumption (P) of bacteria ($\mu\text{g C g}^{-1} \text{ soil day}^{-1}$) by protozoa with a Type III sigmoid functional response (Hunt et al., 1984):

$$P = VA / [1 + (K/B)^n] \quad (1)$$

where V is the relative maximum feeding rate of protozoa on bacteria (day^{-1}); A is amoebal C ($\mu\text{g C g}^{-1} \text{ soil}$); B is bacterial C ($\mu\text{g C g}^{-1} \text{ soil}$); K is the half saturation constant for protozoan consumption of bacteria ($\mu\text{g C g}^{-1} \text{ soil}$); and n is a nondimensional shape parameter set to 10. This equation gives near maximum grazing when $B \gg K$, but reduces grazing disproportionately when $B < K$.

It was possible to produce a model that fitted the data observed for the clay loam soil. The simulated $\text{CO}_2\text{-C}$ evolved during the first 12 days was mainly due to glucose addition ($171 \mu\text{g C g}^{-1} \text{ soil}$) and internal cycling of C in the soil ($160 \mu\text{g C g}^{-1} \text{ soil}$). During this interval, bacterial C uptake was 5.5-fold greater than the initial bacterial C pool size (Rutherford and Juma, 1992c). Protozoan addition to soil directly increased total $\text{CO}_2\text{-C}$ evolution by 11%

and mineralized twice as much $\text{NH}_4\text{-N}$ as did bacteria during the first 12 days compared to the protozoa-minus treatment. Mineralization of C and N was rapid when bacterial numbers were increased as a result of glucose addition.

Model performance was also tested with the data set obtained for a sandy loam (Rutherford and Juma, 1992d). We lowered the value of the half saturation constant for the protozoan consumption of bacteria from 175 to 50 ($\mu\text{g C g}^{-1}$ soil) and initialized the state variables with measured initial values for this soil. The $\text{CO}_2\text{-C}$ evolved during the first 12 days was mainly due to glucose addition ($180 \mu\text{g C g}^{-1}$ soil) and internal cycling of C in the soil ($130 \mu\text{g C g}^{-1}$ soil). During this interval, C inputs into bacteria were 14-fold greater than the initial pool size. Protozoa increased total $\text{CO}_2\text{-C}$ evolution by 16% and increased net $\text{NH}_4\text{-N}$ mineralization 3.5-fold compared to the protozoa-minus treatment. Mineralization of C and N was more rapid in the sandy loam than in the clay loam when bacterial numbers were increased due to glucose addition.

The model represented the hypothesis put forward by Rutherford and Juma (1992b) that protozoa more effectively grazed bacteria and mineralized more C as $\text{CO}_2\text{-C}$ and N as $\text{NH}_4\text{-N}$ in a coarse-textured soil than in a fine-textured soil, and was a good way to check the internal consistency of several hypotheses about pools and flow rates and provided additional information on the flows of C and N within various soil pools which could not be determined experimentally. For example, in fine-textured soil the largest ^{15}N pool on Day 12 was the active pool, followed by the stable, bacterial, amoebal and soluble pools (Fig. 3). However, in the coarse-textured soil the largest ^{15}N pool was the stable pool, followed by the active, amoebal, bacterial and soluble pools (Fig. 3). Thus, soil texture affected bacterial–protozoan interactions and the dynamics and partitioning of added N.

Mathematical modelling of the microcosm experiment provided a rigorous link between soil organism populations and the processes in the C and N cycles. Differences in soil texture, which impacted on bacterial–protozoan interactions, were incorporated into the model to predict the effect that texture had on protozoan-induced mineralization of bacterial N. The link between the biological interactions within different physical environments in the two soils (Soil B, a clay loam; Soil C, a sandy loam) were explored with a Type III sigmoid functional response of bacterial grazing by protozoa (eq. 1). Reduction of the half saturation constant (K) for the protozoan consumption of bacteria effectively adapted the model from a soil in which bacteria were well protected from protozoan predation (Soil B) to a soil in which they were not well protected (Soil C).

Future research

Several physical (texture, structure, pore size distribution), chemical (cation exchange capacity, mineralogy) and biological (mobility of organisms,

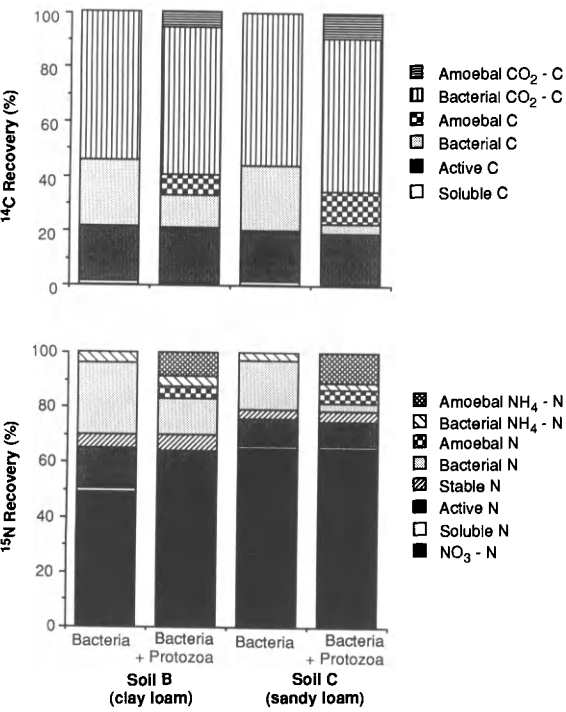


Fig. 3. Model simulation of ¹⁴C and ¹⁵N distribution on Day 12 for Soil B and Soil C, with and without protozoa.

types of fauna) factors affect *K*. Future research to develop an explicit link between these factors and predator–prey activities in soil is needed to link the impact of soil porosity on mineralization of C and N by soil organisms.

HOW TO ASSESS INTERRELATIONSHIPS BETWEEN SOIL MICROBIAL–FAUNAL ACTIVITY AND SOIL ORGANIC MATTER THROUGH CARBON AND NITROGEN TRANSFORMATIONS UNDER CONTROLLED CONDITIONS

The amount of N mineralized over a growing season is influenced by many factors such as climate, crop residues, previous management, biological activity and soil type. Mineralization and immobilization of N are continuous simultaneous processes which are mediated by soil organisms (Jansson and Persson, 1982). Microbial biomass serves two major purposes: (i) it is a source and sink of N, and (ii) it is a transformation agent for soil N (Anderson and Domsch, 1980; Paul and Voroney, 1980). Internal cycling of C and N occurs during microbial growth and death in soil. In this section, the approaches used to measure mineralization and immobilization of N in samples from the surface horizons of cultivated soils are presented. The description of micro-

bial faunal interactions is not presented at this scale of resolution because data for soil fauna are less available than those for microbial biomass.

Methods for quantifying pools and dynamics of soil organic matter

The dynamics of soil organic C and N have been studied by four main approaches over the past 100 years: (i) acid hydrolysis; (ii) classical humic fractionation techniques; (iii) particle size fractionation; and (iv) the kinetic approach, which categorizes heterogeneous organic materials by their decomposition rates and disregards their chemical identity. The kinetic approach, developed by Paul and Juma (1981), advanced the pioneering work of Jansson (1958) and Jenkinson (1966) by dividing soil organic N into four pools: (i) microbial biomass (mainly bacteria and fungi); (ii) non-microbial active N; (iii) stabilized fraction; and (iv) old organic matter. This approach has been successful at predicting mineralization-immobilization turnover of N in soils (Juma and McGill, 1986). The advantage of this method of organic matter study is that the chloroform fumigation method for determination of microbial C and N (Jenkinson and Powlson, 1976) can be used to directly link the decomposer food web with elemental cycling. Use of ^{14}C and ^{15}N tracers allows the dynamics and partitioning of amended C and N to be followed amongst various pools in the plant-soil system (Paul and Juma, 1981). The advantages of these methods and representative examples have been reviewed by Juma and McGill (1986).

Use of simulation models

Many simulation models have been developed to describe the N cycle in soil (Frissel and Van Veen, 1981). Internal cycling of C and N occurs during microbial growth and death in soil. The size and activity of microbial biomass is explicitly defined in most mechanistic models describing C and N dynamics. The role of soil fauna in communiton, grazing and nutrient cycling may be defined or implied (Hunt and Parton, 1986). The relation of activity of soil fauna either directly to the decomposition of residues and soil organic matter or indirectly through alteration of soil porosity have not been well quantified. Therefore, many models do not include faunal activity to describe the internal cycling of C and N.

A systematic analysis of the models based on the mechanisms used and the accuracy of description of different processes have not been made. This is partly due to lack of scientific knowledge and partly due to the different approaches used to study the C and N cycles in soil.

We compared the mineralization and immobilization submodel of the Phoenix model (McGill et al., 1981) and the Tramin model (Juma and Paul, 1981) using a common experimental data set. A comparison of features of

the models and results of model performance are given by Juma and McGill (1986). The simulated net changes calculated by both models were similar to the experimental data but the inputs and outputs from various pools were markedly different. A summary of results is shown in Fig. 4. The microbial growth calculated with the Phoenix model is higher than that of Tramin because all organic N was directly incorporated into the bacterial and fungal biomass. In contrast, turnover of NH_4^+ calculated using the Tramin model was greater than that of Phoenix because all N was mineralized outside the microbial cells and only a small portion of NH_4^+ was taken up to meet the needs of microbial biomass. The rest of NH_4^+ was nitrified. Nitrate uptake was calculated in the Tramin model and was zero because the soil was in net mineralization conditions and enough NH_4^+ was present to meet microbial needs. Microbial biomass decomposition and net mineralization were similar in both models. The models showed that net mineralization was almost one-half of actual mineralization.

Most simulation models are validated against different data sets; however, a rigorous examination of the mechanisms used to describe the dynamics of C and N through different pools has not been made. In addition, only a few models have simulated the flow of ^{14}C , ^{12}C , ^{14}N and ^{15}N . In spite of simulating three isotopes (^{12}C , ^{14}N and ^{15}N) with Phoenix and Tramin models, we found marked differences in the inputs and outputs from various pools. A synthesis of basic knowledge and documentation of inputs and outputs from various pools used in different models is urgently needed.

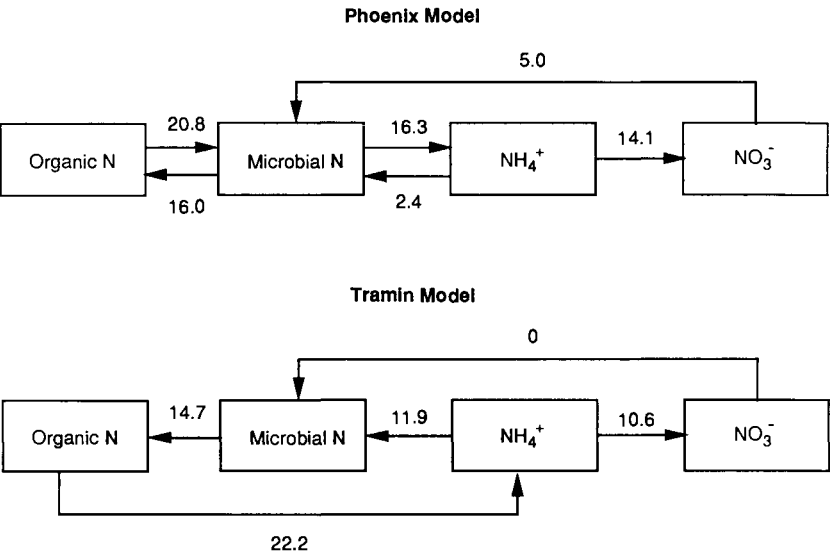


Fig. 4. Comparisons of N flux (g/m^2) through various pools of Weirsdale loam soil calculated with Phoenix and Tramin models.

Description of the effect of texture on C and N cycling in simulation models

Recently, the effect of clay content has been incorporated in soil organic matter models (Van Veen et al., 1984; Parton et al., 1988; Verberne et al., 1990). Clay content influences the amount of the "protected biomass" in soil (Verberne et al., 1990) and also the availability of substrates to soil organisms. Clay content also affects the porosity of soil and the niches for microorganisms and soil fauna. The lack of information on the dynamics of soil porosity in the presence of larger fauna and plants has hindered modellers to incorporate porosity as a dynamic variable in the simulation models.

Future research

Although mineralization and immobilization processes have been studied in a wide variety of soils, the effect of porosity on microbial and faunal activity has not been directly incorporated into the simulation models. Mathematical representation of porosity is needed to further understand the dynamics of processes in soil C and N cycle.

HOW TO ASSESS INTERRELATIONSHIPS BETWEEN SOIL MICROBIAL-FAUNAL ACTIVITY, SOIL ORGANIC MATTER AND CROP PRODUCTIVITY THROUGH CARBON AND NITROGEN TRANSFORMATIONS UNDER FIELD CONDITIONS

The five kingdoms of organisms (Monera, Fungi, Protista, Animalia, Plantae) are represented in soil (Price, 1988). The numerous roles of soil organisms have been described (Mitchell and Nakas, 1986; Edwards et al., 1988; Paul and Clark, 1989). Root inputs and litter inputs from higher plants provide most of the energy and carbon inputs for the free-living heterotrophs of the decomposer food web. The activities of the soil bacteria and fungi, in their search for energy and carbon (C), are largely responsible for the mineralization-immobilization turnover of soil N (Jansson and Persson, 1982). Even though soil fauna are responsible for less than 10% of soil respiration (Petersen and Luxton, 1982) they may play an important role by regulating the size and activity of the microbial biomass (Elliott et al., 1984), in C and N mineralization (Coleman et al., 1983) and in the alteration of soil porosity (Pawluk, 1986). Fauna may have a relatively larger direct contribution to N mineralization than C mineralization because the C/N ratio of fauna is high compared to the C/N ratio of their food (i.e. microbes).

Approaches used to study the effect of soil type on soil biota and organic matter dynamics

In order to understand the impact of soil structure/texture on soil biota and organic matter and plant productivity, field experiments were conducted on

TABLE 2

Soil climate classification (Government of Alberta and University of Alberta, 1969), climatic normals for 1951–1980 period (Atmospheric Environmental Service, 1982a–c) and soil characteristics at Ellerslie and Breton, Alberta (Crown and Greenlee, 1978)

	Ellerslie	Breton				
Soil-climate	Cryoboreal/subhumid	Cryoboreal/subhumid				
<i>Climate</i>						
Annual precipitation (mm)	452	547				
Mean max. temperature July (°C)	22.4	21.2				
Mean min. temperature July (°C)	9.6	8.8				
Mean frost-free days	109	80				
Degree days > 5°C	1230	1060				
<i>Soil</i>						
	Horizon			Horizon		
	Ap	Ae	Btl	Ap	Ae	Bt ₁
pH	6.4	6.2	6.3	6.0	5.7	5.2
Organic C (g/kg)	52	9	3	14	4	4
Organic N (g/kg)	5.0	1.0	0.4	1.2	0.4	0.4
CEC (cmol (+)/kg)	400	160	170	150	100	250
Bulk density (Mg/m ³)	0.9	1.1	1.2	1.1	1.3	1.6

a Typic Cryoboroll mapped as the Malmo silt loam series at the Ellerslie Research Station (53°25' N, 113°33' W) and on a Typic Cryoboralf mapped as the Breton loam series at the University of Alberta Breton Plots (53°07' N, 114°28' W). Typic Cryoborolls are naturally endowed with a thick mollic epipedon which has granular structure, high nutrient and base status, and neutral pH. In contrast, Typic Cryoboralfs are acid, leached, degraded soils with an eluviated horizon. These soils are important for cereal production in Alberta and represent two extremes of natural fertility, crop productivity and biological, physical and chemical properties. Climatic characteristics and soil properties are given in Table 2.

To determine the effects of soil type on the dynamics of soil organic matter, soil microbes and soil fauna, microplots (20 cm diameter, 30 cm depth) were set out at Ellerslie and Breton. Detailed information on microbial, faunal, and plant interactions, and on C and N cycling which has been reported by Ruthersford and Juma (1989a, b) and Dinwoodie and Juma (1988a, b). The highlights of these papers are summarized below.

Although microbial C (g m^{-2}) was greater at Ellerslie, microbial C made up a larger proportion of soil C (mg g^{-1} soil C) at Breton (Table 3). Protozoa (No. m^{-2}) were not significantly different between sites but were greater at Breton when expressed relative to soil C (No. g^{-1} soil C). The magnitude of

TABLE 3

Absolute and relative comparisons of selected variables in 0–10 cm depth at the two sites

Variable	Site		Significance of site effect ^a
	Ellerslie	Breton	
<i>Absolute (per m² basis)</i>			
Soil C (g m ⁻²)	5529	2376	***
Microbial C (g m ⁻²)	45.9	26.4	*
Protozoa (No. m ⁻²) × 10 ⁹	108	211	ns
Nematodes (No. m ⁻²) × 10 ⁵	30.2	6.9	**
Acari (No. m ⁻²) × 10 ³	42.7	19.7	\$
Collembola (No. m ⁻²) × 10 ²	43	9	\$
CO ₂ -C evolved (g m ⁻² 10 days ⁻¹)	10.3	10.7	ns
<i>Relative (per g soil C or microbial C)</i>			
Microbial C (mg g ⁻¹ soil C)	8.2	11.1	*
Protozoa (No. g ⁻¹ soil C) × 10 ⁶	19	88	*
Nematodes (No. g ⁻¹ soil C) × 10 ²	5.5	2.9	ns
Acari (No. g ⁻¹ soil C)	7.7	8.3	ns
Collembola (No. g ⁻¹ soil C)	0.80	0.36	ns
CO ₂ -C evolved (mg g ⁻¹ soil C 10 days ⁻¹)	1.9	4.5	***
CO ₂ -C evolved (g g ⁻¹ microbial C 10 days ⁻¹)	0.23	0.41	**

^a\$=p<0.10, *=p<0.05, **=p<0.01, ***=p<0.001, ns=not significant.

protozoa may be overestimated due to methodological problems. Acari, collembola and nematodes were greater at Ellerslie when expressed on an area basis (No. m⁻²); however, did not significantly differ between sites when expressed relative to soil C (No. g⁻¹ soil C). Soil C at Breton supported proportionally greater microbial C and protozoan populations and equal nematode and microarthropod populations compared to Ellerslie (Table 3). Ten-day incubations of non-fumigated soil showed that CO₂-C evolved was greater at Ellerslie (120 µg g⁻¹ soil) than at Breton (97 µg g⁻¹ soil); however, there was no significant difference in CO₂-C evolution when expressed on an area basis (g m⁻²) between soils (Table 3). CO₂-C evolution was greater from Breton soil samples when expressed as a proportion of soil C (mg g⁻¹ soil C) or as a proportion of microbial C (mg g⁻¹ microbial C) (Table 3).

Microbial N, non-microbial organic N (NMON) and mineral N made up a larger proportion of soil N at Breton compared to Ellerslie (Table 4). Net N mineralization was not different between soils when expressed as a proportion of microbial N; however, it was greater in soil from Ellerslie when expressed as a proportion of NMON. Net N mineralization was greater in soil from Breton when expressed as a proportion of total soil N. Net ¹⁵N mineralization did not significantly differ between soils when expressed as a proportion of soil ¹⁵N (Table 4).

A hypothesis was proposed by Rutherford and Juma (1989b) that the food

TABLE 4

Absolute versus proportional N pool sizes and net N mineralized in 0–10 cm. Soil from Ellerslie and Breton

Variable	Site		Significance of site effect ^a
	Ellerslie	Breton	
<i>Absolute (per m² or g soil basis)</i>			
Soil N (g m ⁻²)	456	206	***
Microbial N (g m ⁻²)	5.5	4.1	ns
NMON ^b (g m ⁻²)	46.1	66.2	ns
Total mineral N (g m ⁻²)	0.98	0.83	ns
Net N mineralized (μg g ⁻¹ soil 10 days ⁻¹)	10.4	7.2	*
Net ¹⁵ N mineralized (μg 100 g ⁻¹ soil 10 days ⁻¹)	4.2	3.0	ns
<i>Proportional (per unit N basis)</i>			
Microbial N (% of soil N)	1.2	2.0	**
NMON ^a (% of soil N)	10	32	**
Total mineral N (% of soil N)	0.21	0.40	**
Net N mineralized (mg g ⁻¹ microbial N 10 days ⁻¹)	17	20	ns
Net N mineralized (mg g ⁻¹ NMAN 10 days ⁻¹)	2.4	1.5	*
Net N mineralized (mg g ⁻¹ soil N 10 days ⁻¹)	0.20	0.39	**
Net ¹⁵ N mineralized (% of soil ¹⁵ N 10 days ⁻¹)	4.8	3.2	ns

^a* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = not significant.

^bNon-microbial organic N.

web was more active at Breton compared to Ellerslie in 1986. Differences in detrital trophic web structure affect C and N turnover in the soil and the size and activity of the active N pools. Expressed proportionally, high C activity and a large decomposer food web (Table 3) kept a larger proportion of soil N in the soil organisms at Breton compared to Ellerslie (Table 4). Rapid C turnover and greater microbial and faunal activity resulted in a greater proportional net N mineralization rate at Breton.

Results from other experiments showed that the amount of N mineralized over 40 weeks under laboratory conditions from surface soil samples from Ellerslie was almost three times that of Breton; however, the proportion of total N mineralized from Breton was higher than that at Ellerslie (Fig. 5). These results support the hypothesis put forward by Rutherford and Juma (1989b). More research is needed to quantify the sizes and rate constants of biota and soil organic matter fractions undergoing mineralization in different soils.

Interaction between crops and soils and above ground productivity

The above ground productivity under conventional and alternative cropping systems at Ellerslie and Breton was studied by growing: (i) barley, (ii)

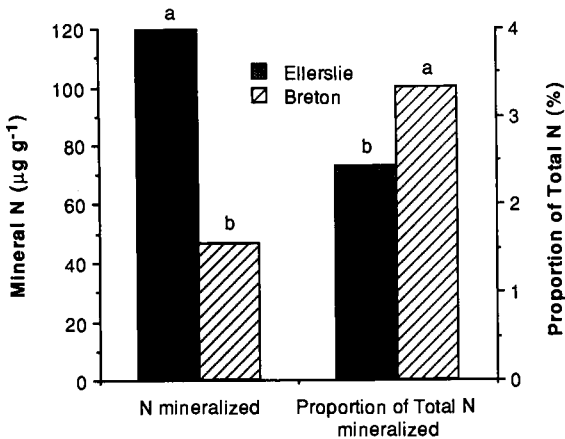


Fig. 5. Amount of N mineralized from surface soil samples from Ellerslie and Breton expressed on an absolute and relative basis.

barley intercropped with field pea, and (iii) faba bean continuously from 1986 to 1988 inclusively (Izaurre et al., 1993). Under similar levels of management, the 3-year average C yield over all crops was higher at Ellerslie (316 g m^{-2}) than at Breton (282 g m^{-2}). The average barley C yield from fertilized plots at Breton was only 10% lower than that at Ellerslie (Fig. 6). In contrast, barley yield differences of up to 50% were observed between sites on unfertilized plots at Breton (M. Nyborg, pers. commun., 1992) emphasizing the importance of N inputs if comparable barley yields are to be attained. In the case of the intercrop, the yield differences between sites was 14% (Fig. 6). In contrast, the 3-year average C yield of faba bean (382 g m^{-2}) was higher than at Ellerslie (328 g m^{-2}). Without soil-water stress, the faba bean/*Rhizobium* symbiosis is capable of fixing high amounts of dinitrogen at Breton. Using enriched ^{15}N isotope techniques, Gu (1988) reported up to 200 kg ha^{-1} of N fixed by faba bean at Breton.

The amount of N uptake by barley represents N uptake from fertilizer N and from N mineralized from soil organic matter. Assuming half of N uptake in the barley crop is from fertilizer N, the amount of N which was mineralized and taken by crop (3.8 to 5.5 g m^{-2}) represents microbial and faunal activity. In addition to fertilizer and soil organic matter, biological N fixation in annual legumes increased above-ground yield by 1.5- to 2-fold compared with barley. Except for faba bean, annual fertilization with N and P as well as prevailing climatic conditions, masked to some extent the influence of inherent soil properties on crop yield and demonstrated the importance of hierarchical constraints (e.g., irradiance, temperature > water > nutrients) on crop productivity under cryoboreal conditions.

The results of this 3-year study show that in spite of inherent differences in

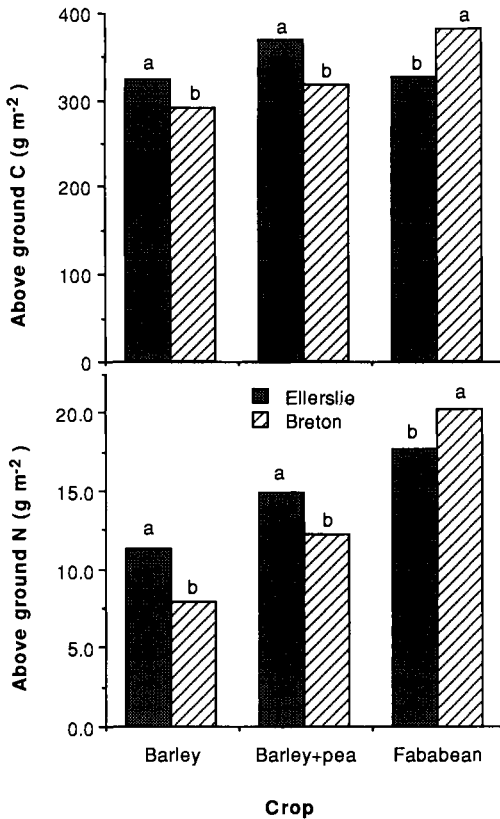


Fig. 6. Average above-ground C and N yields of three crops at Ellerslie and Breton.

soil properties (organic C and N, soil texture and structure, pH, etc.), yields of barley, and barley–field pea intercrop fertilized with N and P at Breton were no more than 26% lower than those obtained at Ellerslie. At Breton, a special soil–climate–plant interaction resulted in a 16% higher faba bean yield than that obtained at Ellerslie. A rather favourable water regime at Breton, may have contributed to these results.

Under field conditions, the primary productivity is affected by a number of variables. Fertilizer N is rapidly partitioned in the soil–plant system and some is lost by leaching and denitrification (Rutherford and Juma, 1989b, 1992e). The soil supplies N and water to the crops over the growing season. However, these constraints can be removed through management. The biological effects are generally masked by management practices in managed agroecosystems.

Future research

In highly managed agroecosystems, the role of organisms to mineralize nutrients from soil organic matter is partially replaced by use of external inputs.

Activities of organisms and their effect on mineralization immobilization processes has to be studied in reduced-input or in impoverished systems. This approach can yield knowledge on the biological potential of different soils.

HOW TO ASSESS INTERRELATIONSHIPS BETWEEN PLANT PRODUCTIVITY AND SOIL STRUCTURE/TEXTURE THROUGH CARBON AND NITROGEN TRANSFORMATIONS UNDER CONTROLLED AND FIELD CONDITIONS

Roots are important for plant anchorage, water and nutrient uptake, storage of carbohydrates, synthesis of growth regulators and input of organic carbon into the soil. An understanding of these functions and processes involved are critical for improving plant performance and soil quality. Knowledge of root growth and distribution underpins our ability to understand root-shoot strategies for water and nutrient utilization, and organic matter dynamics. These strategies are important in determining the ability of a crop to successfully tolerate periods of excess or limited water, or nutrient stresses. The input of C into soil affects nutrient cycling and nutrient availability, microfloral and faunal activity, and soil structure formation. These in turn control the amount of plant available nutrients.

Effect of different crops and cultivars on root/shoot ratios, root lengths and root mass

Information on below-ground productivity is important for constructing ecosystem budgets (Hansson and Andrén, 1986) and studying plant-soil interactions. At Ellerslie and Breton, root mass of fababean at all depths was higher than barley (Izaurre et al., 1993). Existing differences in chemical, physical properties between soils, however, appeared not to induce differences in root production during 1987. Root/shoot ratios for the species followed the same order on both sites: faba bean (0.32 and 0.25) and barley (0.12 and 0.12). On both soils, between 80 and 90% of barley- and faba-bean-root biomass was present in the 0–14 cm soil layer. Root biomass reflected crop characteristics rather than soil influences on root growth (Izaurre et al., 1993).

Results from an experiment in which four barley cultivars were grown in a Typic Cryoboroll at Ellerslie over a period of 2 years showed that there was a significant cultivar by growth stage interaction for root mass and root length (Fig. 7) (Xu and Juma, 1992). Root length dynamics did not follow trends in root mass dynamics through the whole growing season. Root length at ripening stage was significantly lower than root length at heading stage but there was no significant difference between root mass at heading and ripening stages. Perhaps this was mainly due to the decomposition of fine roots during the

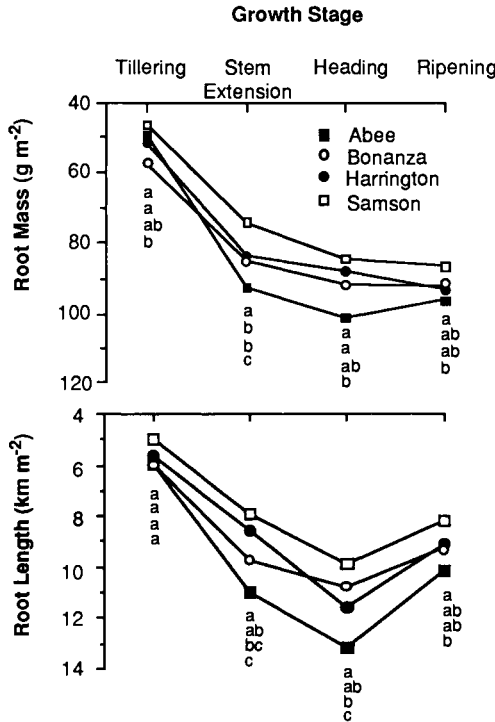


Fig. 7. Total root mass and root length of four barley cultivars at various growth stages over 1989 and 1990. The means of the cultivars bearing the different letters are significantly different in root mass or root length at the given growth stage ($p < 0.05$) and refer to the magnitude of these variables from the lowest to the highest.

late period of the growing season. Thus, decomposition of fine roots affect root length more than root mass.

The results of this study showed that: (1) shoot and root mass of Harrington and Bonanza were not different from Abee and Samson but those of Abee were significantly greater than those of Samson indicating that the below ground input of organic matter could be a function of specific cultivar; (2) root mass increased rapidly until the heading stage, while the shoot mass increased at a higher rate than roots between the heading and ripening stages producing a widening of shoot/root ratios with time; and (3) decomposition of very small roots may have contributed to a more rapid decrease in root length than in root mass after heading stage (Xu and Juma, 1992). In order to assess the complete impact of root inputs into the soil ecosystem, more information is needed for other types of root-released organic materials such as root exudates, sloughed-off root cells and mucilage.

Effect of different barley cultivars on distribution of photosynthetically fixed ^{14}C

The input of C through roots affects mineralization and immobilization processes and controls the activity of soil biota. Although the standing root mass at harvest may be only 10% of shoot mass, a quantity equal to about 3- to 4-fold of standing root mass C at harvest is released into the soil over the growing season in form of root material, exudates and other soluble products (Sauerbeck and Johnen, 1977). Thus up to 33% of total C fixed by photosynthesis is used to build and maintain the root system. Van Veen et al. (1991) summarized previous work and reported that 60 to 90% of the total carbon assimilated by arable crops was stabilized in different pools of the crop-soil system and 10 to 40% of that was released from the roots into the soil. Estimations of annual input of carbon into soil by a growing crop ranged from 900 to 3000 kg ha⁻¹. Large variations exist with plant species, cultivars and development stages and environmental conditions (Van Veen et al., 1991).

The transformation of labelled carbon in two barley cultivars/soil systems in field conditions over the growing season using pulse ^{14}C labelling technique was measured by Xu and Juma (1992). We hypothesized that cultivars which produce more soluble C rather than standing root mass would stabilize more C in microbial biomass and soil organic matter. For Abee, root ^{14}C decreased significantly with time over the growing season, but for Samson it increased from tillering to stem extension stage, and then decreased till the ripening stage (Fig. 8). At the stem extension and heading stages, root ^{14}C of Samson was significantly higher than that of Abee. The ratios of shoot ^{14}C /root ^{14}C increased significantly with time over growing season for both cultivars, but the rate of increase was significantly higher for Abee than for Samson. At the tillering stage, the ratios of shoot ^{14}C /root ^{14}C for the two cultivars were similar to each other, but from the stem extension stage, it increased faster for

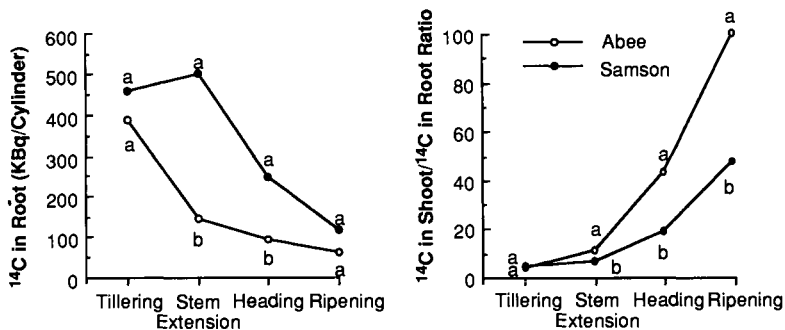


Fig. 8. Root ^{14}C and the ratios of shoot ^{14}C /root ^{14}C for two barley cultivars over growing season. The means of the cultivars bearing the different letters are significantly different at the given growth stage ($p < 0.05$).

Abee than for Samson. At the ripening stage, the ratio of shoot ^{14}C /root ^{14}C of Abee was almost twice as much as that of Samson.

Our results of ^{14}C activity in soil were obtained 15 days after labelling and could reflect the C transformation characteristics of the barley–soil systems under steady-state conditions. The total C in root of Abee was greater than that of Samson, but ^{14}C activity in shoot and root of Abee was lower than that of Samson. The ratios of total C in shoot over total C in root at ripening stage of Abee was about 1.1-fold higher than that of Samson, but the ratio of shoot ^{14}C /root ^{14}C at the same time was 2-fold higher for Samson than for Abee. This indicated that the photosynthetically fixed C was transferred and cycled faster in Samson than in Abee. The C translocation rate below-ground for Samson was also higher than for Abee. Overall, soil ^{14}C under Abee was significantly lower than that under Samson at the stem extension and heading stages but there were no significant differences at the tillering stage and ripening stages. These results suggest that the partitioning of C by different cultivars have to be considered in assessing the impact of translocated C on the amount of organic matter in soil. The decomposition of fine roots and exudates occurs at a microscale, therefore soil pore structure has to be considered in understanding the dynamics of various processes in the rhizosphere.

Comparison of destructive and in situ methods for quantifying root length and root mass of different crops

The evolution of the minirhizotron from a qualitative to a quantitative tool enable one to compare the dynamics of root growth using destructive (core or monolith) and nondestructive methods (Upchurch, 1991). The studies by Böhm et al. (1977) and Köpke (1982) were the first attempts in comparing root study methods for soybean and oat root systems under field conditions. However, comparisons with the recently improved minirhizotron technique (Upchurch, 1987) are required for other crops and soils. We compared the minirhizotron, core and monolith methods in measuring root length distribution for barley [*Hordeum vulgare* (L.) cv. Bonanza] and faba bean syn. broadbean [*Vicia faba* (L.) cv. Herz Freya] under greenhouse conditions.

Linear regression analyses were used to describe the relationships for root mass density (RMD) and root length density (RLD) between different methods (Table 5). Each depth represented paired values since CVs in individual observations were large (Heeraman and Juma, 1993). Mean RLD by the minirhizotron was excluded in the top 10 cm layer in the regression analyses because estimates of RLD were lower than the monolith method. In general a positive linear relationship existed between the methods. Correlation coefficients were significant ($p < 0.01$) between monolith and core methods in barley and fababeen for both RMD and RLD.

TABLE 5

Relationships between the minirhizotron (MR), core (C) and monolith (M) methods in estimating root mass (mg cm^{-3}) and root length (cm cm^{-3}) density for barley and faba bean

Crop	Depth (cm)	Regression equation ^a	r^b
<i>Root mass density</i>			
Barley	0-70	$M = -0.01 (\pm 0.02) + 0.73 (\pm 0.29)C$	0.75*
Fababean	0-70	$M = -0.02 (\pm 0.01) + 1.49 (\pm 0.10)C$	0.99**
<i>Root length density</i>			
Barley	0-70	$C = -2.31 (\pm 0.90) - 0.73 (\pm 0.76)MR$	0.39ns
	10-70	$C = -0.81 (\pm 0.88) + 1.70 (\pm 0.70)MR$	0.77*
	0-70	$M = 1.85 (\pm 0.80) - 0.95 (\pm 0.68)MR$	0.53ns
	10-70	$M = -0.64 (\pm 1.07) + 0.99 (\pm 0.85)MR$	0.51ns
	0-70	$M = -0.15 (\pm 0.42) + 0.68 (\pm 0.29)C$	0.73*
Fababean	0-70	$C = 0.53 (\pm 0.50) - 0.01 (\pm 0.42)MR$	0.01ns
	10-70	$C = 0.14 (\pm 0.19) + 0.19 (\pm 0.16)MR$	0.52ns
	0-70	$M = 0.66 (\pm 0.66) - 0.08 (\pm 0.56)MR$	0.06ns
	10-70	$M = 0.14 (\pm 0.23) + 0.19 (\pm 0.19)MR$	0.46ns
	0-70	$M = -0.09 (\pm 0.05) + 1.31 (\pm 0.08)C$	0.99**

^aFigures in parentheses are standard errors.

^bCorrelation coefficients are significant at: * = $p < 0.05$; ** = $p < 0.01$; ns = not significant.

Comparison of monolith and core methods

The correlation coefficients obtained between the monolith and core methods for root mass and root length densities were significant. The CV of root length and root mass of faba bean using the monolith method was lower than the core method. The CV of root mass of barley using the monolith method was lower than the core method; however, an opposite trend was found for root lengths. This suggests that the consistency of measurement using destructive sampling methods depends on the type of crop. In barley, root length estimates by the monolith method were lower in the upper 40 cm soil depth compared to the core method but were higher at all depths in faba bean. Hence, the monolith method was more consistent for faba bean than for barley. This is not surprising since the CV of root length for the whole profile obtained with the monolith method was higher in barley (42%) than in faba bean (24%). In comparison to faba bean, barley roots do not exhibit a random variation in diameter but fall in discrete bands related to the order of the root.

Hackett (1968) estimated that the fine branch roots or primary laterals (diameter 0.2–0.5 mm) can constitute up to 80% of total root length in the root system of barley. Loss of these fine roots from washing a larger sample in the upper 40 cm soil layer may explain lower RLD estimates compared to minirhizotron and core methods. Also, incomplete separation of live roots from organic material, loss in detection of fine roots from overcrowding and

the resolution limit of the video-image analyzer can introduce errors during automated root length measurements (Cunningham et al., 1989). These errors were much smaller in faba bean than in barley because of a coarser and less dense root system. Vogt et al. (1989) also concluded the monolith to be unsuitable for fine root data collection compared to the core method.

Root distribution can be quantified using destructive and non-destructive sampling methods. The results from this study has shown that the core and monolith methods can be used for different crops. However, the minirhizotron technique still needs to be calibrated against different methods especially in the top 10 cm layer. Destructive sampling still remains the method to quantify root growth in this layer which is a zone of intense microbial and faunal activity.

Use of micromorphological methods to study effects of biota and crops on soil microstructure

Micromorphological investigations of cultivated soils at Ellerslie and Breton revealed marked differences in soil microstructure and fabric. The Typic Cryoboroll from Ellerslie had an inherent ultra fine and very fine granular microstructure that was very resilient to change through management practices. The different structural arrangements of these primary granular units reflected variable degrees of coalescence and disruption by both natural and human activity. The basic units of structure were approximately 50–70 μm in diameter. Some of the units are made up entirely of humic material, some comprise strongly complexed humic substances and clay and some contain admixtures of fine silt. Their genetic origin is likely very complex since some units are well defined fecal pellets of microarthropods, some appear to reflect biochemical alteration of plant protoplasm and some reflect characteristics associated with physical processes of wetting and drying and freezing and thawing. Some of the smaller units have been incorporated into larger units through ingestion by larger arthropods. These fecal pellets may be discrete or strongly welded to form larger units ranging up to 1 mm or more in size and imparting to the soil a very fine and fine granular character. The smaller primary units of structure are also partially fused through compaction by tillage and root growth as well as by swelling through wetting and frequently intergrade to a spongy microstructure. Disruption through tillage in the upper soil layers breaks up these soil masses to form fragments and clods of highly variable size and shape. Drying below the cultivated layer results in shrinkage and the formation of intersecting vertical and horizontal fracture planes to impart a ultra and very fine blocky microstructure to the soil mass. In zones where the soil mass is relatively denser and moisture movement is somewhat impeded well developed horizontal and parallel planar voids develop through cycles of freezing and thawing and impart a platy microstructure to the soil

mass. Throughout this physical activity the integrity of the primary units is generally retained.

The Typic Cryoboralf at Breton did not have similar primary units developed within their microstructure. Soil materials are highly porous in most cases with strongly interconnected vughs and metavughs that form a spongy microstructure. Few discrete micropeds are evident but frequently, near the surface, weak and moderately developed irregular shaped peds were discernible. Similar features were previously observed to occur through freeze thaw processes and appear to reflect an extreme point in the development of interconnected vughs. Spongy microfabrics also result from earthworm action. The strongly welded fecal material is generally much denser than the adjacent matrix material and the resulting granular units are coarse to very coarse granular in size. In zones where vughs are not interconnected the soil materials characteristically have a vughy microstructure that intergrades to blocky, sub-angular blocky, or platy.

Cultural practices showed variable affects on microstructure. The roots of fababeans showed the greatest amount of compaction as reflected in the development of vughy and spongy microfabrics within a 1 to 2 cm zone. The greatest amount of earthworm activity was observed in the barley plots of both soils where the strongly welded casts imparted a coarse granular and spongy microstructure to the ingested materials. The best ultrafine and fine granular microstructures were observed in the pea plots where considerable microarthropod activity was evident (S. Pawluk, pers. commun., 1992)

Future research

To date, knowledge on shoot/root ratios, C inputs from different crops and cultivars is empirical. The partitioning of C in crops, the thickness of roots and amount of exudates are controlled by many factors (Van Veen et al., 1991). The thin-section techniques using florescence dyes may be one way to increase our understanding of soil biota, soil organic and inorganic components at a micrometre scale (Postma and Altemuller, 1990). Future research is needed to understand the synlocalization and synchronization of plant, microbial, and faunal activity in soils. An insight into effects of porosity on microbial, faunal and plant activity is needed.

CONCLUSIONS

Interrelationships between soil texture/structure, soil biota/organic matter and crop production occur at different scales of resolution. At finer scales of resolution, experiments can be conducted to understand specific processes, however the detailed information has to be translated to the next level of resolution. Our work showed that these approaches can be used to link microbial

and faunal activities to cycling of C and N in soils with different textures. At coarser scales of resolution, for example under field conditions, many other processes become important. Therefore, simple relationships between the numerous parameters have to be identified and modelled.

Porosity is controlled by a number of physical (wetting and drying, freezing and thawing), chemical (type and amount of clay and sesquioxides), biological (microbial, faunal and plant activity) processes and management practices (tillage, crop grown). Mathematical representation of porosity is needed to further understand the dynamics of processes in soil C and N cycle.

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Gas, water and solute transport in soils containing macropores: a review of methodology

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ABSTRACT

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The physical, chemical, and biological characteristics of soil bodies, interacting with weather, management practices, and chemical and biological processes, influence the movement of gas, water, and solutes in cropland ecosystems. The activities of soil biota, especially through their effects on soil porosity, impact on these properties and processes. This paper reviews methods for measuring flow into and through the soil, with emphasis on the techniques being developed to assess the importance of biological activity on the flow characteristics of soils. The discussion of methods used to characterize gas, water, and solute transport in soils addresses conditions under which their use may or may not be appropriate and the need for further methodology development.

INTRODUCTION

In the early 1900's and before, field and laboratory descriptions of soils consisted largely of visual observations of properties such as soil color, wetness, stone content, texture, and the presence or relative abundance of cracks and biopores which were suspected of having major influence on the movement of water, nutrients, and gases.

Advancing technology of the 20th century brought increased capability to quantify unseen soil properties and to vastly increase the number of observations or measurements of potentially interesting soil parameters. By the 1950's, computers gave soil scientists the capability to solve in seconds or minutes mathematical equations that had previously required months or even years to complete.

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Soil physicists of the 1960's used computer simulation to predict the movement of water and chemicals. Sophisticated models, based on Darcy's Law and Philip's solution of a diffusion equation (Philip, 1957), simulated infiltration, evaporation, and water and chemical uptake by plants from multi-layered soils. Each modeled soil horizon was assigned a unique set of water content–pressure–conductivity relations that described the soil as a porous, conducting body made up of homogeneous, isotropic layers.

For nearly two decades, water movement in soils was calculated based on models that were driven and constrained by soil moisture tension. With these models, flow in large, non-capillary sized pores could not be considered. By the 1980's, the documented preferential movement of water and solutes in macropores encouraged the development of field and laboratory techniques for characterizing preferential flow paths and elicited the further development of models that included macropore flow components.

The effects of macropores on transport phenomena have been neglected because macropores often represent only a small proportion of the total porosity and because of the difficulty in quantifying their contribution or influence. Many of the traditional methods of assessing transport phenomena and porosity rely on small cores or disturbed samples and characterize pores within the capillary size-range. Where macroporosity may be important, new procedures must take into account the non-capillary behavior of macropores and their spatial and temporal variability. In this review we discuss applicability and critically evaluate some recent advances in methodology used to characterize preferential flow and macroporosity in soils.

GAS MOVEMENT

Measurements of gas movement through soil can be made to evaluate direct gas exchange or to characterize the media conditions affecting gas, water, and solute transport. Corey (1986) presented detailed descriptions of laboratory procedures for measuring air permeability under unsteady- and steady-state flow conditions. He discussed theory, limitations, applicability, and devices required for several methods and concluded that none of the reviewed methods were suitable for measuring permeability of undisturbed soils. He also concluded that samples could be accurately characterized, but such values rarely represented field conditions. Kirkham (1947) avoided sample boundary problems associated with laboratory methods by establishing air flow in the field under decreasing air pressure. His method, however, seemed to work only when the soil was relatively dry.

The control of water content while measuring gas flux in the soil is critical. Especially in swelling soils, shrinkage of air flow pathways, as water content increases, may limit permeability of both air and water (McIntyre and Sleenman, 1982). Blackwell et al. (1990) presented a method for measuring air

flow through cores at different water contents and relating them to water permeability. Roseberg and McCoy (1990) developed a macropore air permeameter (Fig. 1) that overcomes many of the limitations cited by Corey (1986). This permeameter employs undisturbed soil samples, controls water tension in the sample, allows for measurement of water content without removal of the sample from the device, eliminates the effects of gravity, and allows for rapid equilibration of water content and tension during air flow measurements in very wet samples. Water tension control is achieved by rotating the sample container which is lined with a wettable, porous plastic of high conductance. The method has proven especially suitable for character-

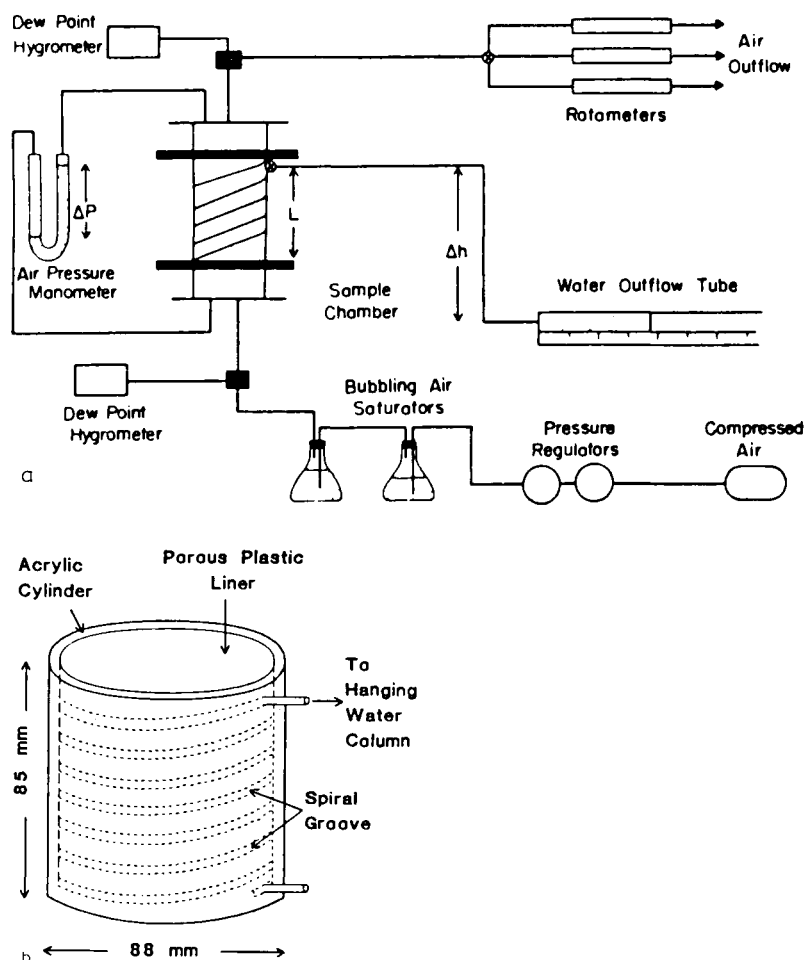


Fig. 1. Macropore air permeameter (a) having sample chamber lined with wettable porous plastic of high conductance (b). (From Roseberg and McCoy, 1990.)

izing continuity of macropores and for detecting crop and tillage effects on macropore extent and geometry (Roseberg and McCoy, 1992).

GAS DIFFUSION

In the air-filled soil pores, gas diffuses along a concentration gradient but flows along a pressure gradient (Ball et al., 1981). Methods for measuring gaseous diffusion have been developed for characterizing porosity and relating it to potential movement of water and solutes (Currie and Rose, 1985). Rolston (1986a, b) reviews advantages and disadvantages of several laboratory and field methods for determining both diffusion and total flux of gases in soils. In any of these methods, especially in the presence of macropores, obtaining an accurate measurement of the concentration of the undisturbed soil gas mixture is difficult to accomplish, but necessary.

Pressurizing or decompressing the sampled gas mixture can influence the concentration of the indicator species. Recently, Mukhtar et al. (1990) presented a method that minimizes potential problems with compression due to air volume changes. Their sampling chamber (Fig. 2) contains a small rubber balloon whose volume is increased by an amount exactly equal to the withdrawn sample. Synchronization of the two processes is accomplished by linking two syringes so that water is injected into the balloon at the same rate that soil air is withdrawn from the chamber (Fig. 3).

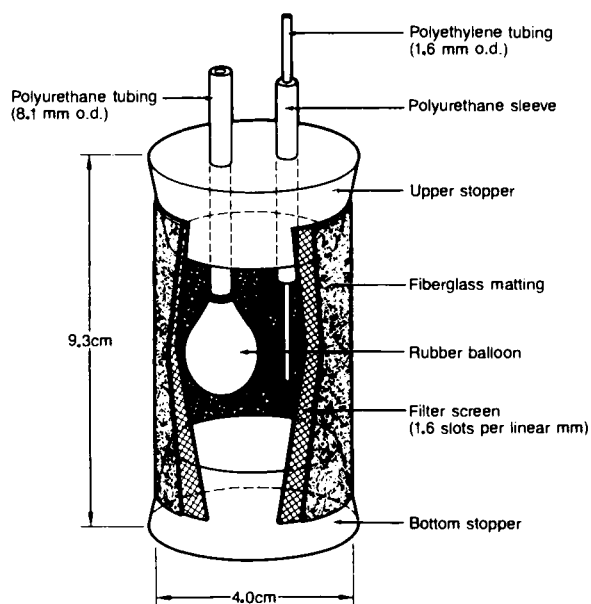


Fig. 2. Soil-atmosphere access chamber. (From Mukhtar et al., 1990.)

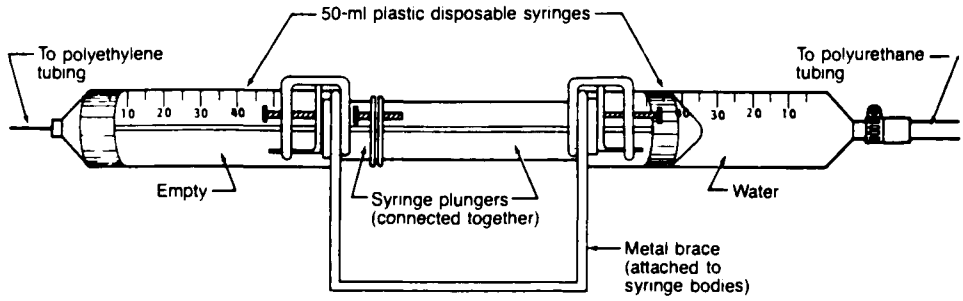


Fig. 3. Dual-action syringe sampling assembly. (From Mukhtar et al., 1990.)

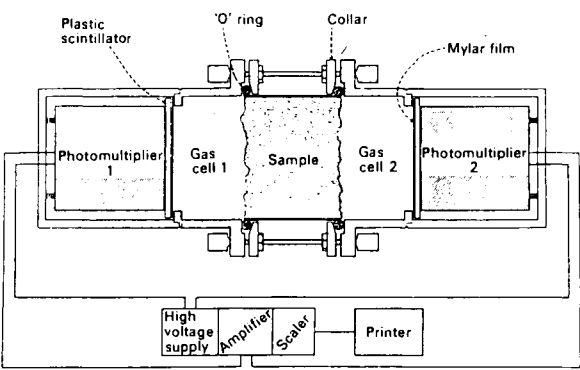


Fig. 4. Apparatus for measurement of gas diffusion and permeability. (From Ball et al., 1981.)

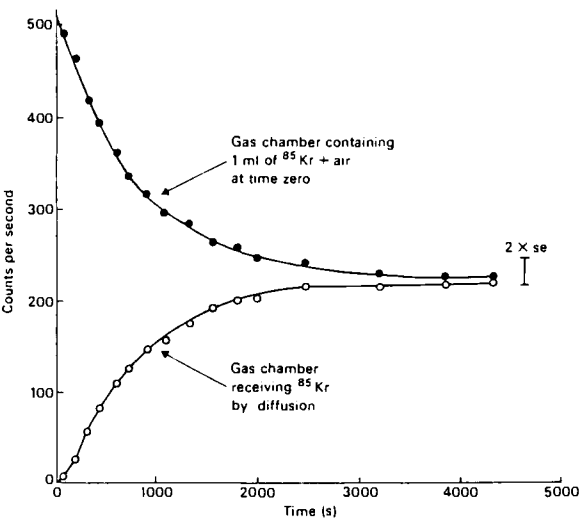


Fig. 5. Typical rate for diffusion of krypton from one chamber into the other. (From Ball et al., 1981.)

Magnusson (1989) designed small volume sample chambers that minimize time required to regain equilibrium with the surrounding soils after sampling has changed internal gas concentrations. His sample chambers could function for long times in the field and worked well both above and below the ground water level.

In the laboratory, Ball et al. (1981) avoided problems with gas sampling by incorporating photomultiplier tubes into diffusion chambers on opposite sides of a soil sample (Fig. 4). The diffusing gas, krypton-85, was injected into one chamber and allowed to diffuse to equilibrium concentration throughout the apparatus. The porosity and permeability characteristics of the soil sample are reflected in the time required for concentrations of krypton to equalize in the two chambers (Fig. 5). The method appears quite appropriate for evaluating the permeability of samples containing large biopores.

Man-made or simulated macropores have been used to help assess the importance of natural macroporosity on transport processes. Ball (1981) measured diffusion and flow of gas through tubes to simulate and model the effects of soil macropores. Healy et al. (1991) monitored the diffusion of helium through soil-filled drums, some of which contained simulated tubular macropores. As Healy et al. (1991) stated, using gas to characterize porosity has several advantages over water: gas will not change water content or structure; it will not cause clays to swell, affecting the macropore network; it is much faster; and an insoluble gas can be quickly flushed out of the sample for repeat testing. As expected, when the evaluated samples contained macropores, the tracer gas was detected almost immediately at the untreated end of the sample.

WATER AND SOLUTE MOVEMENT

A major effect of flora and fauna on soil is reflected in porosity. Almost always, this effect increases pore sizes and variability of porosity. In many cases, traditional soil physical methods cannot detect the presence of a single, continuous biopore large enough to be the dominant flow path through a sample. As described by Smettem and Collis-George (1985b), because a single, continuous 0.3 mm diameter macropore can conduct more water than the rest of a 100 mm diameter sample, pore continuity, in addition to total porosity, must be recognized. Scotter (1978) not only recognized, but modeled preferential flow in theoretical round channels and planar cracks. As noted by Bouché (1971) and Edwards et al. (1988b), the degree of continuity of biopores is often quite different from non-biopores.

Traditional methods for evaluating porosity and transport of water and solute have been reviewed (Beven and Germann, 1982; Nielsen et al., 1986; Wagenet, 1986; Brusseau and Rao, 1990; Coltman et al., 1991). Although for some objectives, the methods do not have to be modified when macropores are present, in other situations novel approaches or modifications are required.

The open, vertical burrows of *Lumbricus terrestris* L. have been implicated in “short-circuiting” of water and solutes (Bouma et al., 1981). This phenomenon is also termed “by-pass flow” and can be characterized by early breakthrough of chemical tracers (Bouma et al., 1983; Bouma, 1991). Colored dyes and other tracers have been used by numerous investigators to confirm that flow can occur in these burrows and other preferential flow paths (Ehlers, 1975; Douglas et al., 1980; Bouma, 1981; Tyler and Thomas, 1981; Germann et al., 1984; Smettem and Collis-George, 1985a; Smith et al., 1985; Everts et al., 1989; Andreini and Steenhuis, 1990).

After Bouché (1971) and Ehlers (1975) demonstrated the tremendous capacity of earthworm burrows for infiltration, Edwards et al. (1979) presented a method to numerically model the effects of hole diameter, depth, and number of holes per unit area on infiltration. This led to increased development of methods for determining the number and size of biopores in the field as determined by management practice.

In the early 1970's, several scientists made direct counts of earthworm hole densities in the field (Lee, 1985). Jongerius et al. (1972) presented a method of characterizing porosity in thin sections using image analysis. Others adapted this technique to characterize biopores, either directly from thin sections (Bouma et al., 1977; Bullock and Thomasson, 1979), from photos of freshly exposed and cleaned soil surfaces at different depths (Edwards et al., 1988a), or from tracings of the macropores drawn on plastic sheets (Logsdon et al., 1990). Scott et al. (1988a, b) presented a similar method for characterizing topology and connectivity of porosity in a cracking clay soil.

Computer assisted tomography (CAT) has recently been used to characterize large biopores in unexposed interior portions of undisturbed samples (Anderson et al., 1988; Warner et al., 1989; Peyton et al., 1992). Figure 6, from Anderson et al. (1990), shows macropores on a gray-scale image and superimposed computed hole perimeters developed using CAT scan techniques. The procedure can be repeated for different positions along the length of a sample to document continuity of continuous pores (such as earthworm burrows) without destroying the sample.

Edwards et al. (1989) developed a method for sampling water flowing in individual *L. terrestris* burrows under natural rainfall conditions in the field. The earthworm burrows were intercepted by excavating horizontally into the walls of a pit. Plastic bottle samplers (Fig. 7) were then placed beneath each burrow to collect water flowing in the burrow at that depth. Conditions affecting infiltration into the soil around the burrow and flow in the burrow above the sampler were not disturbed during installation. A sampler access tube extending to the surface allowed for extraction of the contents of the bottle by vacuum pump after each rainfall event. With 50 individual burrows sampled in a long-term no-till corn field, storm conditions that caused infiltration of water and nitrate transport in the *L. terrestris* burrows were docu-

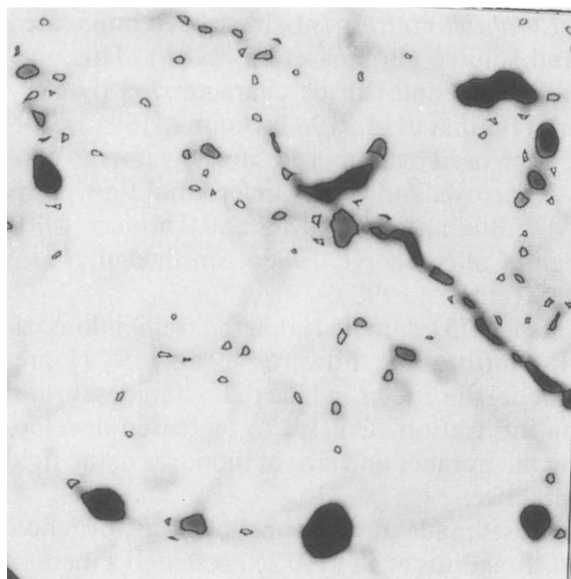


Fig. 6. A computed tomography (CT) gray-scale image of a soil sample containing macropores with superimposed computed hole perimeters. (From Anderson et al., 1990.)

mented. Bouché (1971), Ehlers (1975), Kretzschmar (1982), and Stockdill (1982) reported different methods for measuring water flow in individual wormholes.

A method for evaluating water and solute transport through macropores in 30 cm $\times$ 30 cm $\times$ 30 cm blocks of undisturbed soil was presented by Shipitalo et al. (1990). In the laboratory, the position of all > 2 mm diameter macropores (mostly *L. terrestris* burrows) at the bottom surface of each block was noted (Fig. 8a) before the block was placed on a gridded percolation collector. Simulated rainfall was then applied to the surface of the block causing percolation. Water and solute that moved through the block were collected in 64 separate collectors that could be related to the locations of the earthworm burrows at the bottom of the block (Fig. 8b). Transparent tubing, that led from the 64 collectors at the bottom of the block to sample containers below, allowed for determination of percolation breakthrough times, volumes, and chemical concentrations in the individual samples. The method has been used to evaluate effects of antecedent moisture content, rainfall intensity, initial storm characteristics, and time between chemical application and first rainfall on the movement of water and solute through the preferential flow paths and the soil matrix (Edwards et al., 1992).

There are a number of techniques and devices available to assess the movement of chemicals through the soil profile. The most commonly used methods utilize soil samples, suction cup lysimeters, pan lysimeters, or tile drains.

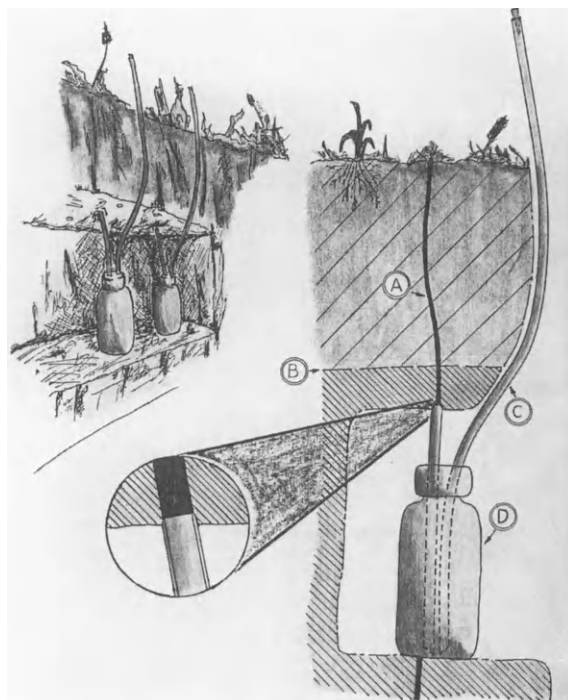


Fig. 7. Earthworm burrow flow sampler, schematic of installation before backfilling: earthworm burrow (A), old plowsole (B), sampler access tube (C), and sample bottle (D). (From Edwards et al., 1989.)

The selection of the most appropriate method depends on the experimental design and degree of accuracy required. In field experiments, where chemical movement in macropore flow is a concern, pan lysimeters should often be the sampler of choice.

Macropore flow can transport small amounts of solute well ahead of the main solute front and far beyond the depths predicted by simple displacement models (Kanchanasut et al., 1978; Thomas and Phillips, 1979; Bouma, 1981; Skopp et al., 1981; Tyler and Thomas, 1981; White, 1985; Douglas, 1986; Van Ommen et al., 1989; Kluitenberg and Horton, 1990; Jarvis et al., 1991). In some circumstances, the movement of even a small fraction of a solute may be of concern. For instance, Steenhuis et al. (1990) pointed out that even 0.1% of a 2 kg ha^{-1} solute application reaching groundwater can result in concentrations in excess of $1 \mu\text{g l}^{-1}$. Under conditions such as these, spatial variability and errors associated with the extraction and recovery of a solute from the soil make the accurate assessment of solute transport using soil cores obtained from the field difficult. In the field, tracer recoveries are commonly $\pm 10\%$ or more of the amount applied. Under these conditions, the movement of an amount of a solute equivalent to a few percent of the

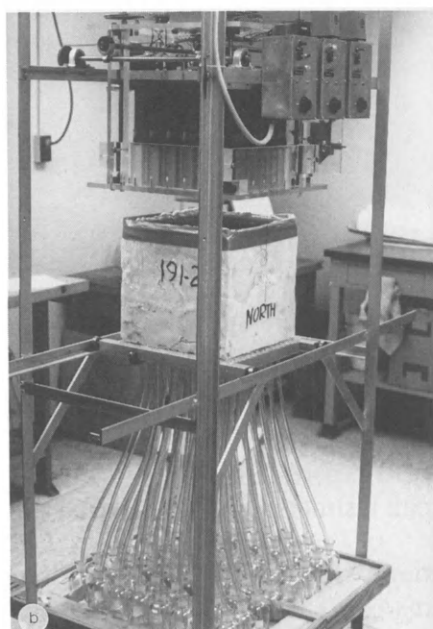
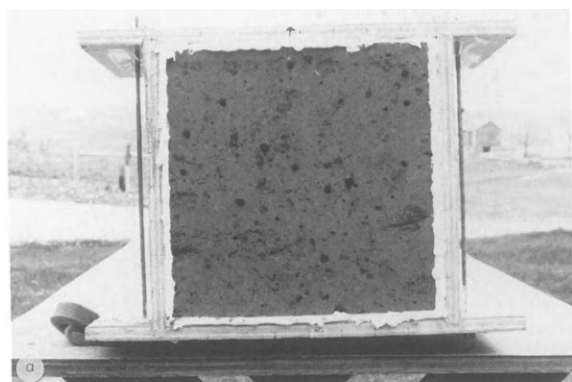


Fig. 8. Bottom of undisturbed soil block showing macropores (a) and rainfall simulator with multiple-segment percolation collection system (b). (From Shipitalo et al., 1990.)

application could not be discerned. Even under controlled laboratory conditions (Priebe and Blackmer, 1989; Shipitalo et al., 1990), significant movement of solute measured in percolate samples could not be detected from soil sample analyses.

Suction cup lysimeters represent an alternative to soil cores in which the soil solution is extracted in situ. There are also a number of drawbacks associated with their use. The cups are generally rather small with low probability that they will be in contact with macropores; consequently, the most rapid

component of the flow may not be sampled. The effect of cup size in relation to macropores is illustrated by Bouma et al. (1982). Similarly, Shaffer et al. (1979) document the inability of suction cups to sample the rapid flow through soils attributable to macropores. Where ceramic cups have been shown to influence quality determinations of some solutes, stainless steel samplers have been required (Smith and Carsel, 1986). Additionally, vacuum must be applied to suction lysimeters in order to obtain a sample. If flow occurs when the samplers are not charged, the contribution of this flow to water quality cannot be assessed. However, the maintenance of cup samplers at a fixed suction level creates unnatural flow conditions and is not recommended (Litaor, 1988). Application of a vacuum also increases the potential that volatile components will be lost (Barbee and Brown, 1986a, b). Thus, spatial and temporal variability in water and solute movement are not adequately accounted for using suction lysimeters.

The use of pan lysimeters, either of a tension-free or suction design, reduces some of the concern over temporal and spatial variability owing to their ability to sample continuously. Also, usable pan lysimeters can be constructed which sample from much larger areas than can be practically accomplished using either soil cores or suction cup samplers. For example, tension-free pan lysimeters used by Jemison and Fox (1991) had a surface area of 0.465 m^2 .

Hall et al. (1989), using tension-free pan lysimeters under no-till and plowed cornfields in Pennsylvania, showed that percolation of water and herbicides was greater under no-till management. They concluded that the lack of tillage provided continuous connections of topsoil macropores to the preferential flow paths of the subsoil and that yearly tillage destroyed macropore continuity, especially that of pores created by roots or macrofauna.

Barbee and Brown (1986a, b) compared suction cup and tension-free pan lysimeters in the field. They noted that the pans generally detected solute movement earlier and solute concentrations were higher than those obtained from suction cup samplers. This led them to conclude that pans are superior to suction cups, especially when preferential flow occurs. One problem encountered with tension-free pan lysimeters, however, is that the soil above them must be saturated before water held in the fine-pore matrix can enter the collection chambers. Theoretically, the build-up of a water mound above the samplers should cause them to shed water, although Tyler and Thomas (1977) were unable to detect any differences in water content immediately above pans compared to the surrounding soil.

In Georgia, a similar comparison of suction cups and tension-free pan lysimeters produced opposite results (J. Hook, pers. commun., 1991). In sandy soils that contained few large biopores, he found higher concentrations of nitrate in samples collected from suction cups than from pans. The suction cups also collected higher concentrations when the nitrogen was spread on the soil surface and subsequently moved into the soil by rainfall than when the nitro-

gen was applied in irrigation water. The application method had no effect on nitrate concentration as collected by tension-free pan lysimeters. Smith et al. (1990) installed suction cups in a sandy soil and found 3- to 5-fold lower bromide values than from adjacent soil extracts.

The concern that pan lysimeters do not accurately sample water due to the build-up of a water mound should be alleviated by applying tension to the pan. Haines et al. (1982) did this by constructing pan lysimeters from ceramic plates connected to hanging water columns. In some instances, however, the tension pans collected only a fraction of the water collected by nearby tension-free pans, leading them to conclude that pan lysimeters do not provide a meaningful estimate of the quantity of water moving through soil. It is possible that the problems they encountered were due to low hydraulic conductivity of the ceramic plates causing rapid macropore flow to be lost.

Van Grinsven et al. (1988) used suction applied to a porous cloth to pull soil water through a flux meter while measuring unsaturated water flux. Their concern was to match suction at their meter with that at the same depth in near-by undisturbed soil. Suction was successfully monitored and transferred to the collection surface by a micro-processor which, if used at the top of the pan lysimeter, may solve the low conductivity problems encountered by Haines et al. (1982).

Brown et al. (1986) introduced the concept of maintaining suction on pan samplers using wicks. This concept has been refined by Boll et al. (1990) to include the use of multiple wicks. Possible concerns over their use include the fact that the matric potential of the wick is a function of flow rate and the wicks are subject to hysteresis (Boll et al., 1990). Nevertheless, in a field test, Boll et al. (1991) found that greater amounts of percolate and Br tracer were recovered by multiple-wick pan lysimeters than by tension-free pan lysimeters. This led them to conclude that wick lysimeters were superior for determining groundwater loading of solutes. In one direct side-by-side comparison, wicked lysimeters captured 71% of a surface Br application while only 7% was recovered from similar pans without wicks. The reliability of wick lysimeters installed under field conditions for extended time periods has yet to be documented. With any design, the area contributing flow to pan lysimeters is ill-defined.

At a larger scale (Fig. 9), flow of water and solute into the soil and downslope through the soil matrix and in macropores has been studied on isolated blocks in situ (Steenhuis and Muck, 1988; Hornberger et al., 1991). By carefully monitoring sprinkled water input and outflow at different points, preferential flow could be readily documented. Using an instrumented, sloping, forest watershed with subsurface collectors, Watson and Luxmoore (1986) and Wilson and Luxmoore (1988) concluded that under some conditions most of the downslope flow occurred in large macropores representing a small fraction of the soil volume.

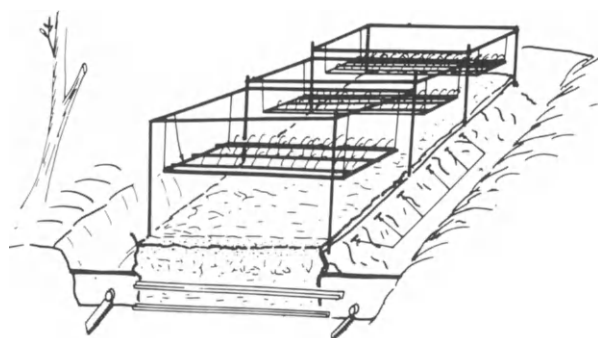


Fig. 9. A 3 m wide by 9 m long semi-isolated hill-side block instrumented to document flow in vertical soil macropores to hillslope processes. (From Hornberger et al., 1991.)

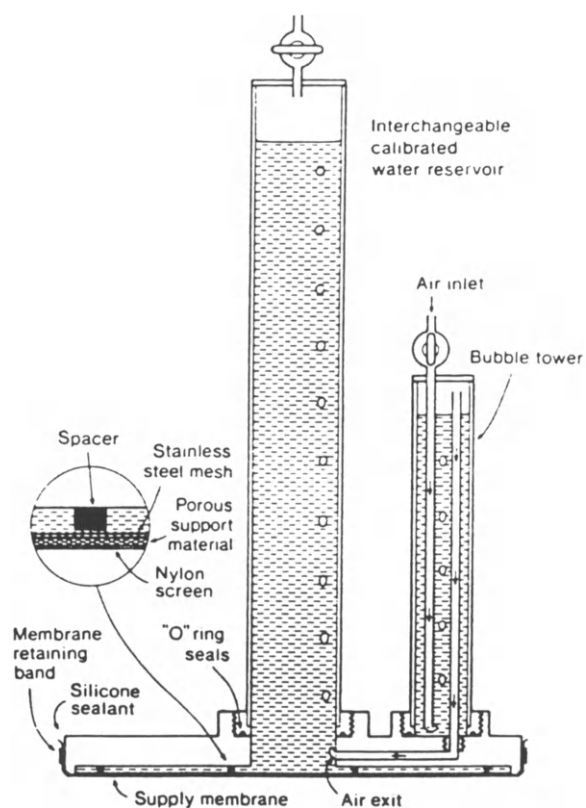


Fig. 10. Tension infiltrometer. (From Perroux and White, 1988.)

To aid in the characterization of this watershed, Watson and Luxmoore (1986) used tension infiltrometers (Fig. 10) that allowed for introduction of water to prepare soil surfaces at slight negative pressures. Theoretically, water

supplied under tension to a soil that is not saturated cannot move into or be conducted in macropores (Dunn and Phillips, 1991b). The development of tension infiltrometers (Dixon, 1975; Clothier and White, 1981; White and Perroux, 1987; Perroux and White, 1988) greatly furthers evaluation of the contribution of different pore sizes to water and solute flux in soils containing macropores (Ankeny et al., 1988; Dunn and Phillips, 1991a).

Macropore flow can also be assessed on a larger scale by analyzing the outflow from tile drains. Early breakthrough of solutes in tile lines approximately 1 m deep has been reported by several researchers (Steenhuis and Muck, 1988). Kladvko et al. (1991) found herbicides in tile effluent after only 20 mm of water had been applied to the surface. They estimated that only 7% of the pore volume was responsible for the early breakthrough of chemical. Richard and Steenhuis (1988) offset the surface chemical application 2 m from the tile trench to prove that the preferential flow was not confined to the backfill. A similar technique used by Everts et al. (1989) showed that both sorbed and non-sorbed tracers moved quickly to the tile lines, indicating macropores or other preferential flow paths were involved.

FUTURE EMPHASIS

Many of the methods and techniques described in the preceding pages result from new and unique developments by the cited authors. In many instances, however, advances in one research area have been made by using and adapting techniques that are standard in non-related fields. Future progress will undoubtedly continue to result from both new and borrowed methods.

Specific examples of techniques that appear to have much broader application for characterizing porosity and the potential preferential flow of air, water, and solutes in macroporous soils are:

(1) The acoustical techniques of Sabatier et al. (1990), who used reflected and transmitted sound waves to characterize porosity, air permeability, and tortuosity to depths of several cm below the soil surface.

(2) The use of brightly colored, small ceramic beads to show how different tillage operations move surface-applied granules into zones of high porosity where they are exposed to preferential flow of infiltrating water (Staricka et al., 1992).

(3) The funnel and plastic tubing environment for testing response of *L. terrestris* to toxic products (Bieri et al., 1989). Although others have used tubes and tubing to simulate macropores, this particular technique has potential application to the study of the effect of earthworm burrow linings on the quality of infiltrating water.

(4) The application of thin-film physics and fractal geometry theories to provide a basis for the observed traditional water content-tension relation (Toledo et al., 1990).

One of the single most important advancements of the last decade in the realm of soil water science has been the general recognition of the importance of preferential flow. The methodology that has developed to document "bypass flow" and its potential transport of industrial and agricultural chemicals has enabled the general public to be widely aware of management practices, agricultural and otherwise, that can influence the quality of surface and sub-surface water bodies.

As our capabilities to recognize and characterize preferential flow increase, it becomes apparent that under many conditions, it may be inappropriate to use average soil or soil horizon properties to predict fate and movement of air, water, and solute. When most of the transport through the soil takes place in a small proportion of the soil volume, as noted by several of the cited authors, average soil properties can hardly be expected to relate well to transport processes. To be able to predict the effects that soil management practices have on the movement of water and solutes, we must be able to characterize the parts of the soil through which most of the movement occurs.

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Simulation modelling as a method to study land qualities and crop productivity related to soil structure differences

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ABSTRACT

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Research on soil biota under natural conditions in the field is complicated because large fluctuations in populations of certain species may occur as a result of natural or man-induced fluctuations in soil physical and chemical conditions. As a result, many experiments have therefore been made in the laboratory under controlled conditions. Though quite valuable, such data may be difficult to extrapolate to field conditions. To better relate biotic data to physical and chemical conditions in the field, expensive long-term monitoring programs can be initiated to collect both types of data.

Physical, and chemical processes in soils can also be characterized by simulation techniques. Simulation requires one-time measurement of some basic and characteristic soil parameters, such as hydraulic conductivity and moisture retention, which are used in a model that calculates water contents in the soil at any given depth and time as a function of precipitation, evaporation and water table levels. Simulation modelling has been applied successfully to calculate water regimes in the soil, for periods of many years. The technique would appear to be particularly appropriate for use in a dynamic physical characterization of different types of soil structure, formed by various soil biota.

Results are presented of a study in a sandy loam soil in the Netherlands with biogenic structures in grassland and a more compact physcogenic structure in arable land. Measured hydraulic conductivity and moisture retention data, and their variability were used in a simulation model to calculate important land qualities for a thirty year period. Land qualities were expressed in stochastic terms by focusing on the probability that certain water and associated air contents and trafficabilities would occur at any given data. Results also reflected the effects of variability of input data. Exploratory modelling was used in this study to estimate effects of an increase of the hydraulic conductivity (which might result from an increase of biological activity), on water and air contents in the soil during the year.

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INTRODUCTION

Increased emphasis in the development of sustainable agricultural systems with lower fertilizer and pesticide inputs and reduced soil tillage and traffic, has stimulated research on soil structure/soil biota interrelationships. Research on soil biota under natural conditions in the field is complicated because large fluctuations in populations of certain species may occur as a result of natural or man-induced fluctuations in soil physical and chemical conditions. As a result, many experiments have therefore been made in the laboratory under controlled conditions. Though quite valuable, such data may be difficult to extrapolate to field conditions. To better relate biotic data to physical and chemical conditions in the field, monitoring programs are being carried out, but they are very expensive because of the need for frequent measurements to adequately characterize changes in the system which are particularly relevant for biotic processes. Also, biologically active systems are quite dynamic and this often presents interpretation problems for physical and chemical monitoring data, since interpretations are based on standard theory which assumes soil materials to be static, and isotropic and homogeneous.

Physical, and to a lesser degree, chemical processes in soils can also be characterized by simulation techniques rather than by continuous monitoring. Simulation requires measurement of some basic and characteristic soil parameters, such as hydraulic conductivity and moisture retention, which are used in a model that calculates water contents in the soil at any given depth and time as a function of precipitation, evaporation and water table levels, if present. Simulation modelling has successfully been applied to calculated water movement in the soil and associated crop growth. The technique would appear to be particularly appropriate for use in a dynamic physical characterization of different soil structures, formed by various soil biota. By determining hydraulic conductivities and moisture retention characteristics, different types of soil structure can be interpreted in a physical manner which, through simulation as discussed, allows a dynamic physical characterization needed to relate different structures to biotic data. Also, simulation allows expressions for periods of time and areas of land, which is a necessary extension to relate soil structure/soil biota interrelationships to soil management procedures, which have to be defined for growing seasons and for parcels of land.

There are, however, limitations to the proposed methodology. Soil structure is considered here as a static attribute with a dynamic character expressed only by simulating the effects of weather conditions over a period of thirty years. Soil structure is, of course, not static. Particularly in arable land, soil structure changes strongly during the years as a result of tillage and traffic. Once a crop has been planted, however, changes are often rather minor. Soil structure is more stable in grassland soils even though trampling by cattle may

induce seasonal changes as well. Finally, the sandy loam texture of the soil being considered precludes significant swelling and shrinkage phenomena. In order to simplify the discussion of variability in space (replicate samples) and time (simulation) we have assumed that soil structure at a given location had one set of static basic hydraulic properties during the years. Prediction of physical processes by simulation uses the existing flow theory which assumes that the soil is composed of isotropic and homogeneous layers or horizons. This may not be the case.

Simulation models need calibration and validation before they can be used for prediction purposes. Some monitoring is therefore required. However, once models are validated their application may cover a very wide range of environmental conditions including variability in space and time, which is crucial to make a connection with practical management systems used in reality. Modelling also allows a sensitivity analysis on the impact of the variability in physical conditions that is due to the variability of the basic parameters and the one due to the soil structure to be characterized. Considering the above discussion, the objectives of this study were:

(1) To physically characterize soil structure formed by certain soil biota by means of dynamic simulation techniques for the soil water regime.

(2) To test the effects of the variability of basic simulation parameters on results obtained.

(3) To interpret results of simulation modelling in terms of land qualities that are important for practical land use.

(4) To use a case study to demonstrate an integrated procedure which dynamically characterizes soil structure (which as such reflects biotic activity), with the objective to use results obtained for defining qualities of land in space and time that are of practical significance.

MATERIALS AND METHODS

Soil structure and hydraulic characteristics

The soils studied were developed in calcareous, medium-textured young marine deposits. They form one mapping unit of the soil map of the Netherlands, scale 1: 50,000, covering about 100,000 ha. These soils occur predominantly in the coastal polders, particularly in the southwestern Netherlands. They are classified as Typic Fluvaquents (Soil Survey Staff, 1975), or as Calcaric Fluvisols (FAO-UNESCO, 1988). Approximately 90% of these soils are used as arable land under a rotation of potato, sugar beet and cereal, mainly winter wheat. The remaining 10% is in use as pasture. Calculations are restricted to the potato crop as part of the crop rotation (cf. Van Lanen et al., 1987). We expect that other crops will react in a comparable manner to dif-

ferences in soil structure, although potatoes are particularly sensitive to structural differences.

The soil has a loamy A horizon. With depth, the clay content decreases slightly, and below about 80 cm the profile consists of locally laminated, loamy, very fine sand. In this study, special emphasis is placed on the layer below the Ap horizon, extending from approximately 30 to 40 cm below the surface. Bulk densities for the ploughpan in arable land were 1.70 kg/m^3 , in pasture they were 1.45 kg/m^3 . The structure in the ploughpan was described as compact apedal with a few channels with a diameter of 1.5 mm and sometimes with a platy structure. In pasture, the structure was weak subangular blocky with peds smaller than 5 mm. For further details about the soils, see Kooistra et al. (1985).

The basic unit of study is the volume of soil in which roots of an individual plant develop. In a lateral sense this would correspond approximately with a pedon (e.g. Soil Survey Staff, 1975). In terms of soil structure, a representative elementary volume should contain at least twenty peds (e.g. Wagenet et al., 1991) which corresponds with a volume of approximately six litres of samples for soil physical analyses. Data thus obtained are extrapolated to field level without additional analyses of spatial variability. To obtain representative data for specific fields (which was not the objective of this study) an additional analysis of the spatial variability of pedons within the field would be needed. The study does not address issues at microlevel as the model considers a bulk root zone and subsoil. Data obtained in this study are representative for horizons at pedon level. If more detailed data are needed on e.g. interaction between roots and individual peds, a more detailed experiment should be designed.

Hydraulic conductivity was measured with six replicates in each of the structure types by the crust test procedure (Booltink et al., 1991) and moisture retention data were measured by desorption in suction cups, as reported by Kooistra et al. (1985).

Model description

The computer simulation model used for this study is the SWANY model, based on the model SWACROP, which includes the soil water flow model SWATRE (Belmans et al., 1983, Feddes et al., 1988) and the crop production model CROPR (Feddes et al., 1978). SWATRE describes one-dimensional (vertical), transient, unsaturated water flow in a heterogeneous soil-root system using the Richards equation, solved numerically by a finite-difference scheme. The soil profile is divided into compartments. The boundary condition at the top of this schematized soil profile is defined as a maximum evapotranspiration flux calculated using daily meteorological data. The bottom boundary condition can be defined by the groundwater table or the water potential or

flux at the bottom compartment of the soil profile. Root water uptake is simulated by a sink term, a function of simulated maximum transpiration and pressure head in the root zone. The crop production model CROPR calculates actual (water-limited) and potential daily crop growth as a function of actual and potential transpiration. SWANY (SWAcrop for a Number of Years) simulates soil water transport and crop production for several years including winter periods, planting or sowing date and crop emergence, i.e. the start of the growing season (Hack-ten Broeke et al., 1990, Van Wijk and Feddes, 1986). Daily meteorological data are needed for all years being simulated to provide the top boundary condition, and at the bottom boundary a flux has to be defined that describes the groundwater regime. Other necessary input data to characterize the soil layers are the water retention and hydraulic conductivity data. These data can be provided by measurements in the laboratory, from field studies or they can be estimated using pedotransfer functions.

Output of SWANY includes moisture contents, air-filled porosities and pressure heads for each soil compartment, and each day, potential and actual transpiration, crop production and groundwater depths. One set of input data results in one set of output. When the range or a distribution of input data is known different sets of input data can be used to assess the possible range of output data as a result of this phenomenon. There are several ways to use stochastic input in a modelling study and a number of such studies have been carried out (e.g. Hopmans, 1987; Wösten, 1990; Petach et al., 1991). For this study a range of water retention curves and hydraulic conductivity curves were available. We did not want to produce stochastic output, but we only wanted to show the possible range of the output parameters. Therefore we decided to run the model for a few combinations of the measured curves, which covered all extremes and thus the whole range.

Computer simulation results of the model ONZAT for four soil structure types of this sandy loam soil were reported by Van Lanen et al. (1987). Differences in workability and aeration, for instance, were prominent for the grassland structure and the primary ploughpan. Therefore, these two structure types were chosen for this study and exactly the same input parameters were used. Other structure types (Van Lanen et al., 1987) were a loosened ploughpan and a secondary ploughpan. These are not discussed in this paper. ONZAT was derived from the model SWATRE and therefore it is assumed that the verification carried out for ONZAT is also valid for SWANY. The crop production submodel in SWANY was not validated in this case, but verification of this model was explored in other studies (Feddes, 1985; Hack-ten Broeke et al., 1990).

Physical land qualities

The model output can be used to derive quantitative expressions for land qualities like moisture supply capacity and trafficability. For specific pur-

poses parts of the model output can be directly used, for instance, crop yields and moisture contents. In this study the land qualities trafficability, aeration, and moisture supply capacity are assessed and productivity is calculated in terms of crop yields. Model output was generated using 30 years of weather data for Dutch conditions.

Trafficability is related to the moisture regime of the soil and is assumed to be directly related to the soil water pressure head (h) at 5 cm below the soil surface (Van Wijk and Feddes, 1986; Bouma and Van Lanen, 1987; Hackten Broeke et al., 1989). When the soil is too wet, in the case of this sandy loam soil when $h > -70$ cm, the soil is considered to be not trafficable. Below this soil-specific threshold value of $h = -70$ cm the soil is considered to be trafficable. (Van Wijk and Feddes, 1986). The question may be raised whether this h value of -70 cm applies to the different treatments. Field observations covered both grassland and arable land and an identical value can be assumed for this exercise. However, careful estimation of critical h values for different structure types deserves more attention in future research. By defining a critical h value the number of trafficable days in any given period of time can be assessed from every daily pressure head value: the value is either higher (non trafficable) or lower (trafficable). A frequency distribution of the occurrence of trafficability for specific periods can be obtained by statistically analyzing all available data for the simulated years.

The aeration status is derived in a comparable way. Here, the air-filled porosity at 5 cm below the soil surface is assumed to determine the adequacy of aeration. Adequate aeration for a potato crop is assumed when the air-filled porosity is higher than $0.1 \text{ m}^3/\text{m}^3$ (Van Lanen et al., 1987). In that case oxygen availability for the roots is considered to be sufficient. Thus, the number of days with adequate aeration can be calculated for any given period, as well as the probability of occurrence of such days for a specific period of time. The moisture supply capacity itself is not assessed in this study, but an indication for the supply capacity can be the moisture deficit, which is determined for every growing season as the difference between potential and actual transpiration. Actual water-limited production is presented, which is simulated by the model.

RESULTS

Water retention and hydraulic conductivity characteristics

Measurements of soil characteristics have been reported by Kooistra et al. (1985). In this paper the characteristics of the soil profiles characterized by the grassland structure and the primary ploughpan were used for simulation runs. In Fig. 1 the range of the moisture retention characteristics for the layer 30–37 cm below the soil surface are presented for the grassland structure (Fig.

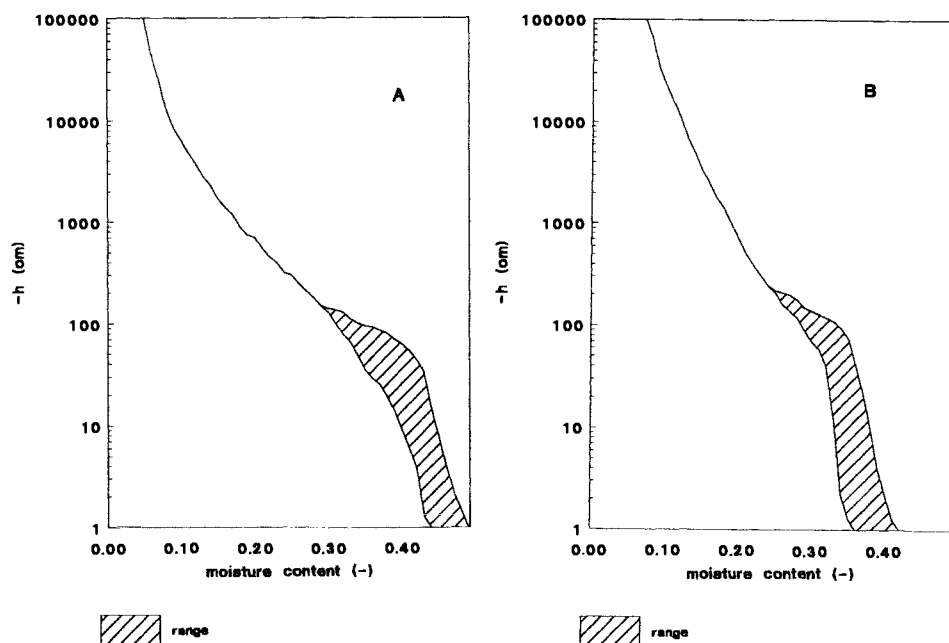


Fig. 1. Water retention curves for the layer 30 to 37 cm below the surface for grassland soil (A) and soil with a ploughpan (B).

1A) and the primary ploughpan (Fig. 1B). The hydraulic conductivity curves for the same depth are presented in Fig. 2. Data are only shown for K near saturation, which most clearly expresses the effects of soil structure. K -data for lower water contents were also measured and used for the simulations. They form one single curve at low water contents. SWANY was run several times as stated above using different curves within the given ranges of Figs. 1 and 2. The range of the curves, used as model input, results in a range of model output. In Fig. 2 an extra conductivity curve is shown, representing a hypothetical situation which could, perhaps, correspond with many worm channels in the grassland structure type. This curve, which does *not* reflect real measured data, was also used for simulation to *illustrate* the exploratory approach of investigating the effect of differences in K -curves.

Simulation results showing temporal variability

A time series of weather data of the meteorological station De Bilt for the years 1951–1980 was used for the simulations with SWANY. Results are presented in Figs. 3–7. The trafficability for the grassland structure and the primary ploughpan are presented in Fig. 3. The curve shows for instance that soil trafficability for the profile with the grassland structure is sufficient in

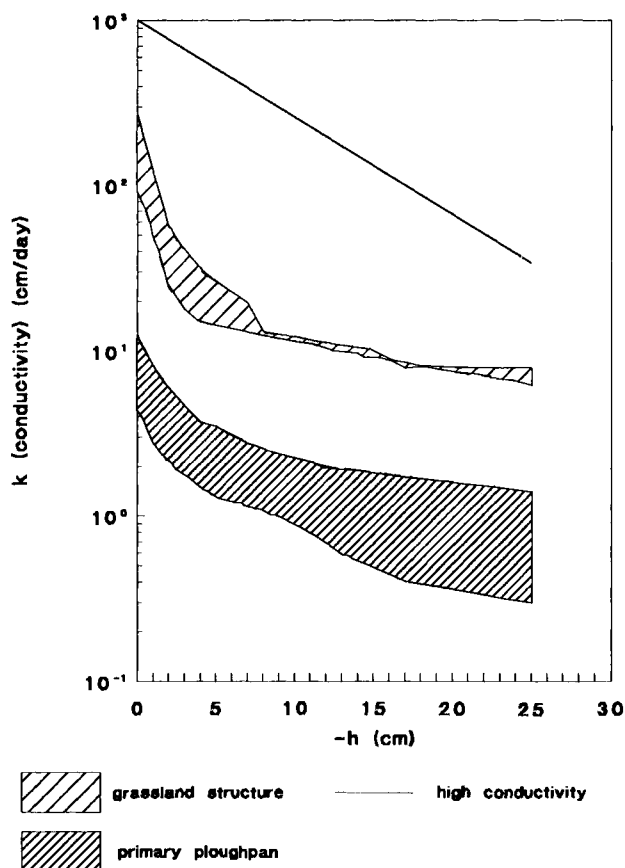


Fig. 2. Ranges of hydraulic conductivity curves for grassland and ploughpan soils. Included is a hypothetical curve which expresses the expected effects of occurrence of macropores, such as worm channels.

approximately 75% of the first ten days of April, whereas it is only 60% in the soil profile with the primary ploughpan. The moisture deficit for this sandy loam soil is very low and never higher than 50 cm during the growing season. Differences between structure types are small and therefore this will receive no further attention. In Fig. 4 the frequency distribution of simulated potato tuber yields is presented. The differences in productivity are also small, but the grassland structure type is slightly better. In 50 out of 100 years the tuber yield will be higher than 14 tonnes dry matter per ha, whereas on the primary ploughpan the yield will exceed only 13.5 tonnes per ha. These diagrams all show average results, but the SWANY output also allows the presentation of more detailed information, like in Fig. 5 for aeration and Fig. 6 for moisture contents. Figure 5 shows the aeration status of the soil for 1–20 April of all hydrological years 1951–1979. In 1951 for instance all 20 days show an air-

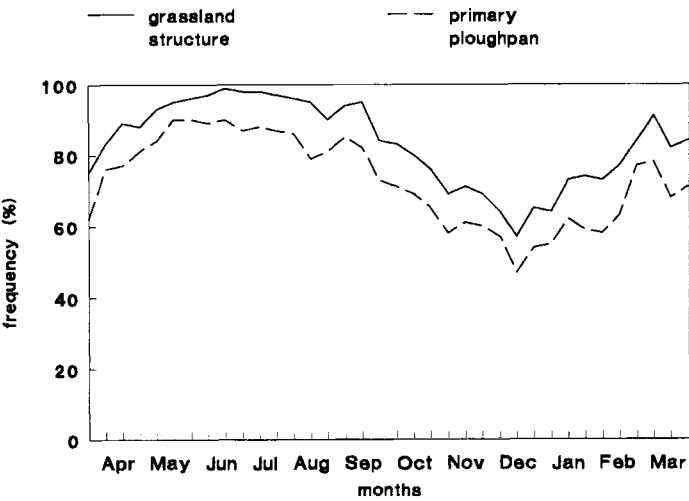


Fig. 3. Cumulative frequency distribution of trafficability for the grassland and ploughpan soil.

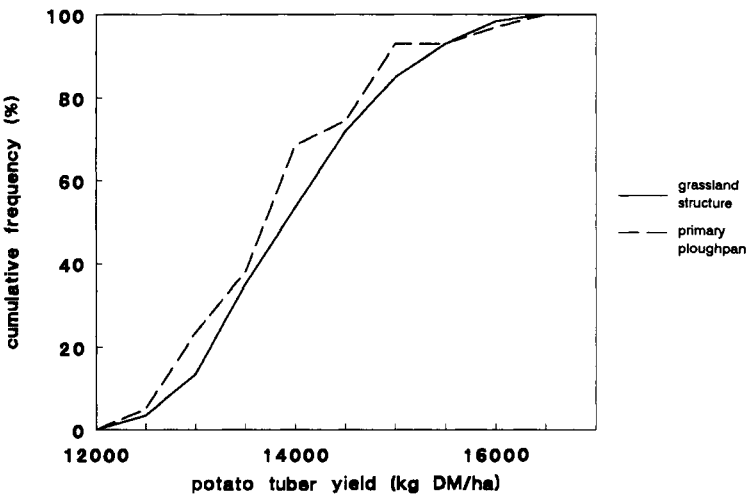


Fig. 4. Cumulative frequency distribution of tuber yield (average curves).

filled porosity at 5 cm below the soil surface of less than $0.1 \text{ m}^3/\text{m}^3$, whereas in 1952 from 16 April onwards the air-filled porosity is higher than $0.1 \text{ m}^3/\text{m}^3$. Such air-filled porosities are also important for processes of denitrification which generally occur under anaerobic conditions. The same type of diagram is presented in Figs. 6 and 7 for moisture contents. The figures show whether the moisture content exceeds or does not exceed $0.35 \text{ m}^3/\text{m}^3$ during

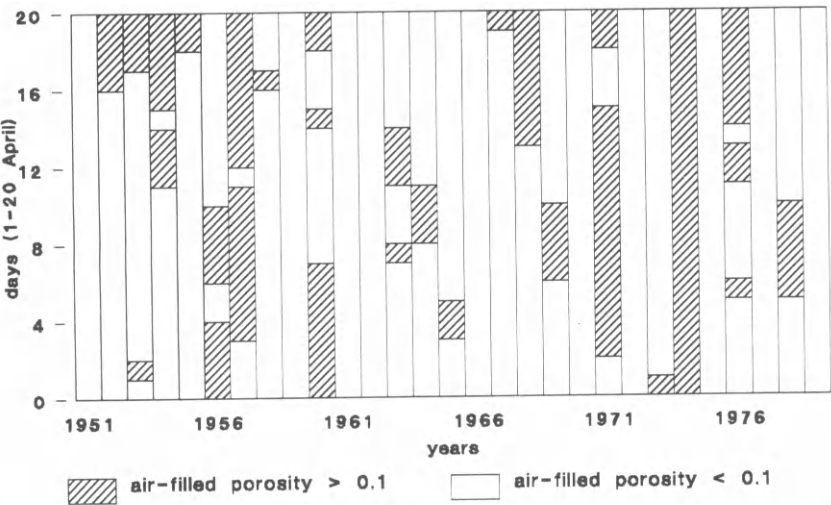


Fig. 5. Aeration at 5 cm below the surface in the grassland soil, for the period 1-20 April.

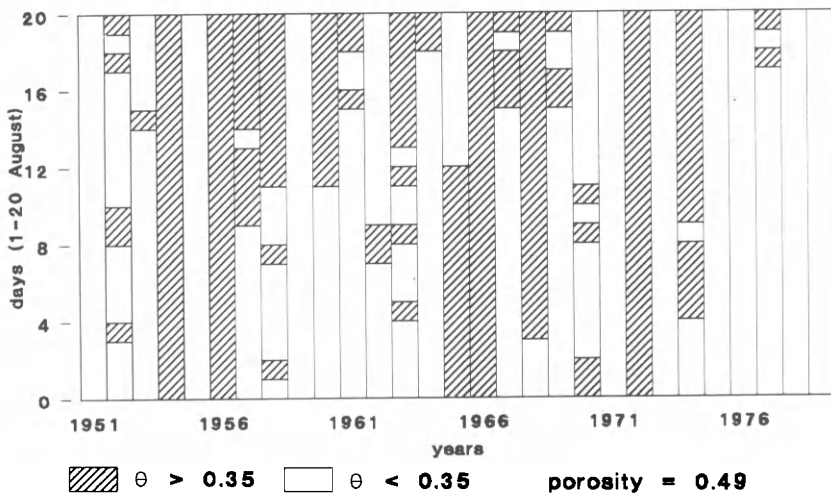


Fig. 6. Occurrence of moisture content (m^3/m^3) at 5 cm below the surface in the grassland soil for the period 1-20 August.

the first 20 days of August. For the grassland structure this value is often exceeded, whilst on the primary ploughpan structure type the moisture content hardly ever gets higher than $0.35 \text{ m}^3/\text{m}^3$. The main reason for this is the difference in water retention characteristics and porosity. At 5 cm below the soil surface the porosity of the grassland soil is $0.49 \text{ m}^3/\text{m}^3$, so that a moisture content of $0.35 \text{ m}^3/\text{m}^3$ means an air-filled porosity of $0.14 \text{ m}^3/\text{m}^3$. At the same time this moisture content corresponds with a pressure head value $h = -170 \text{ cm}$. The soil profile with a primary ploughpan at 5 cm depth has a

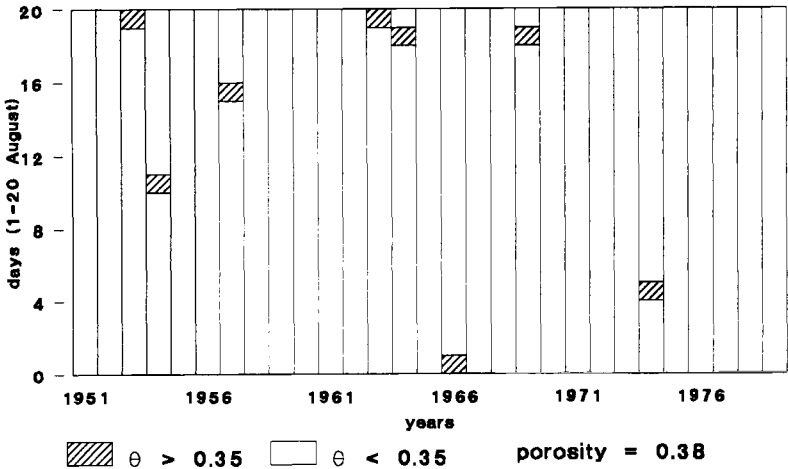


Fig. 7. Occurrence of moisture contents (m^3/m^3) at 5 cm below the surface in three ploughpan soil for the period 1-20 August.

porosity of $0.38 \text{ m}^3/\text{m}^3$, so a moisture content of $0.35 \text{ m}^3/\text{m}^3$ means an air-filled porosity of only $0.03 \text{ m}^3/\text{m}^3$. Furthermore, the corresponding pressure head value is $h = -13\text{cm}$. This moisture content is, of course, not often exceeded. This example clearly shows the importance of the physical soil characteristics and at the same time the problems that can arise when only moisture contents are considered.

Simulation results for variable soil characteristics

For both the grassland structure type and the primary ploughpan structure type simulation runs were performed with water retention and hydraulic conductivity characteristics within the ranges as shown in Figs. 1 and 2. This range in input data results in a range in output, which is presented in Figs. 8–10. On top of these ranges a simulation was carried out for a hypothetical $K(h)$ relation. For both the topsoil and the layer 30–37 cm below the surface (Fig. 2) such curves were used as an alternative for the grassland structure characteristics. The results of this correspond with the curve indicated “high conductivity” in Fig. 2.

Trafficability (Fig. 8) shows no overlap for both types of soil structure, indicating that the number of trafficable days will always be larger for the grassland structure than for the primary ploughpan. The “high conductivity” curve shows an even better trafficability than the grassland structure.

Figure 9 presents the range of frequency distributions for sufficient aeration for both the grassland structure (Fig. 9A) and the primary ploughpan (Fig. 9B). The curve for the “high conductivity” is included in Fig. 9A. Especially during winter time the range of adequate aeration frequency of the

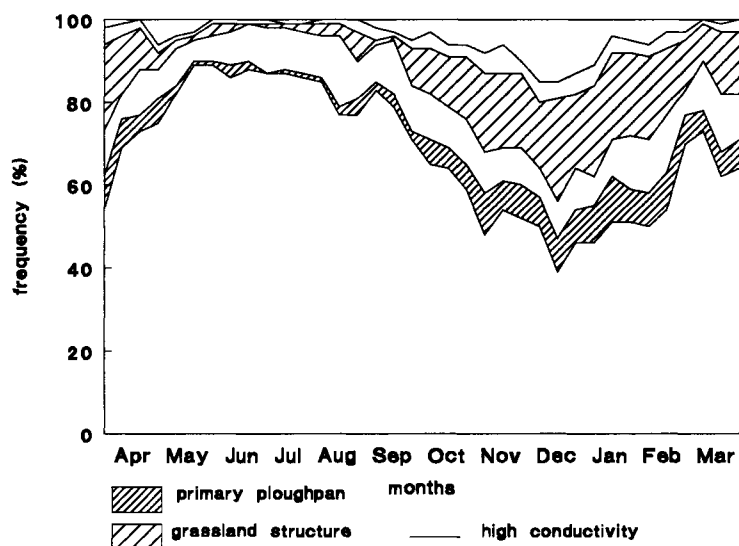


Fig. 8. Cumulative frequency distribution of trafficability for different soil structures associated with grassland, a primary ploughpan and a high hypothetical *K*-curve.

primary ploughpan is often within the range of the grassland structure. During the months June and July, however, the aeration is better for the grassland structure (> 90% of the days show sufficient aeration) than for the primary ploughpan (80–85% of the days). Aeration for the “high conductivity” curve is always slightly favourable.

The ranges in crop production caused by the applied range in input are presented in Fig. 10. It was already obvious from Fig. 4 that differences in potato tuber yields were small, which is confirmed here. The ranges often overlap and a slightly higher production can be found for the grassland structure. The ranges indicate for instance on the one hand that crop production will be less than 14.5 tonnes dry matter per ha in respectively 67–77% and 73–77% out of 100 years for the grassland and primary ploughpan structure, but on the other hand the tuber yield will be higher than 14 tonnes in 40–53% of the years for the grassland structure and only in 23–30% for the primary ploughpan structure.

Exploratory use of modelling

Results of simulations using the so-called hypothetical high conductivity curve (Fig. 2) are presented in Figs. 8 and 9. The effects are relatively minor. One other result of the high conductivity soil characteristics is presented in Fig. 11, which should be compared with Fig. 5. Since there are obviously more

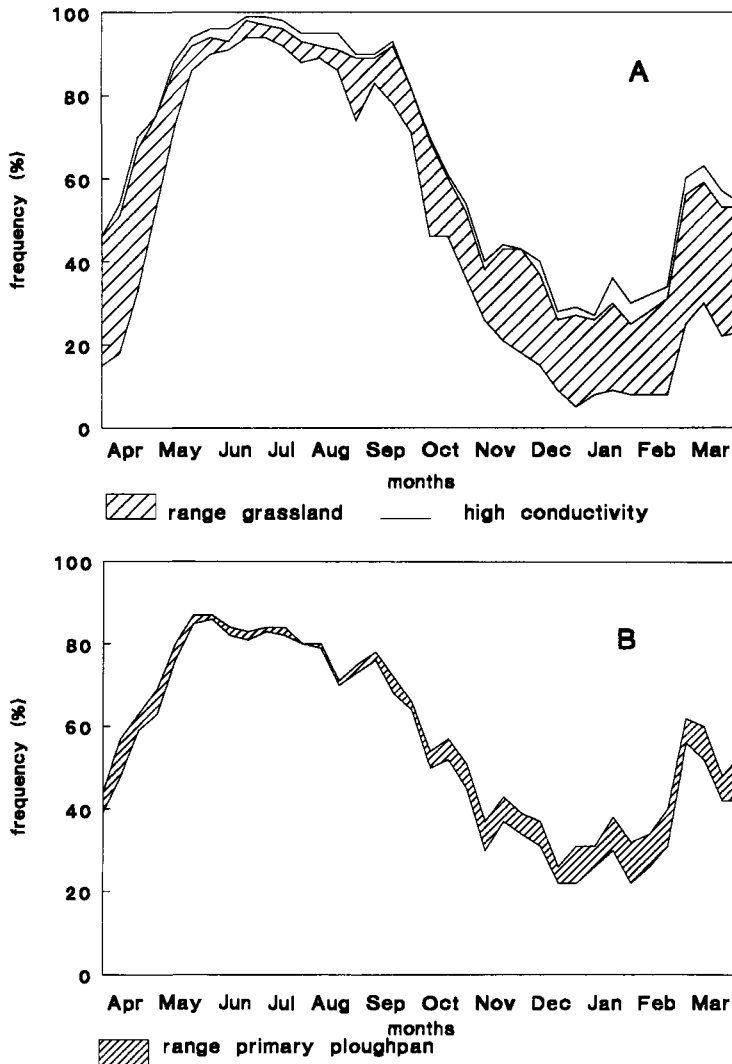


Fig. 9. Cumulative frequency distribution of sufficient aeration for the grassland (A) and ploughpan soil (B) and for soil with a high conductivity.

days with air-filled porosity higher than $0.1 \text{ m}^3/\text{m}^3$ for the high conductivity soil type, high K -values near saturation clearly improves soil aeration.

Use of a hypothetical K -curve in the model-run discussed here, serves as an example of exploratory work, for which simulation modelling is highly suitable. In fact, it would be attractive to start with a desired soil physical condition and then explore with modelling which $K-h$ and $h-\theta$ data would be needed to achieve these conditions. Next, the feasibility of creating structures

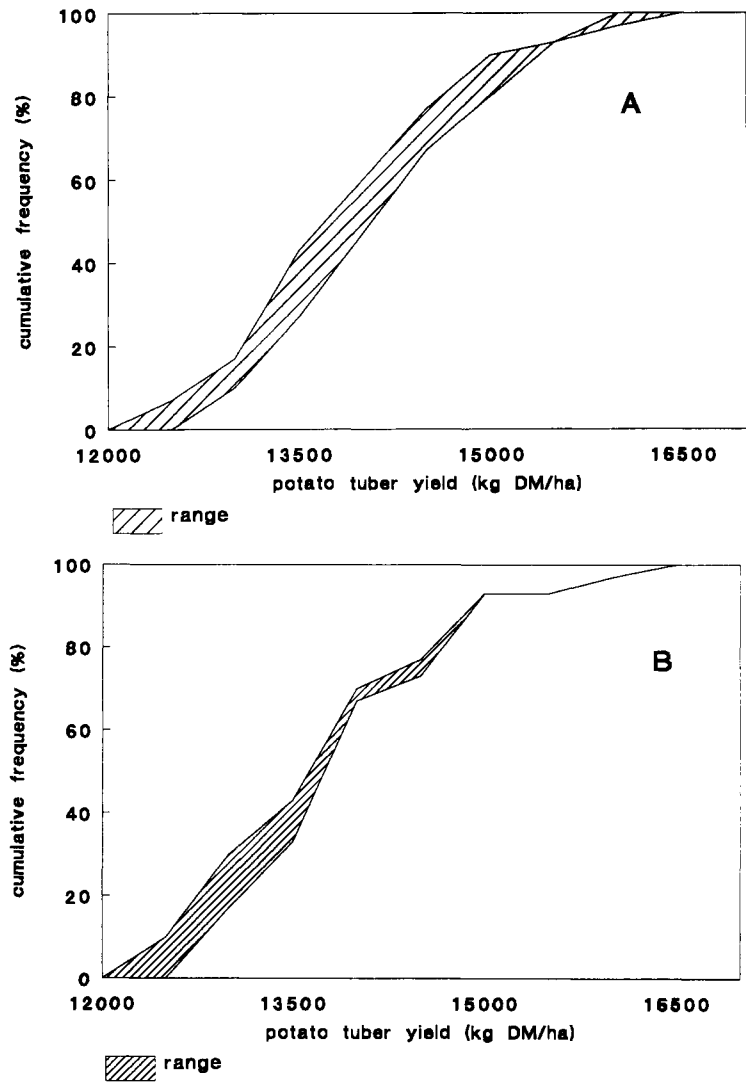


Fig. 10. Cumulative frequency distribution of tuber yield for the grassland soil (A) and the ploughpan soil (B).

with such basic hydraulic properties could be explored. Rather than start directly with expensive experiments, it often pays to explore expected effects by modelling. Other examples could be effects of climatic change on land qualities; effects of lowering water table levels on land qualities, such as water supply and trafficability, and the consequences of compaction on crop yields. Traditionally, agricultural research has been based on experimentation followed by analysis. Modelling allows us to be more explorative.

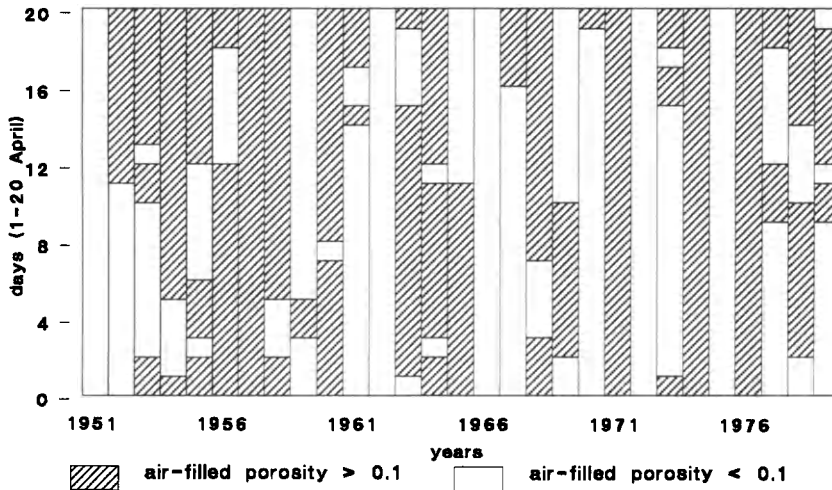


Fig. 11. Aeration at 5 cm below the surface for the hypothetical high conductivity values, calculated for the period 1-20 April. Refer to Fig. 5 which shows similar values for the grassland soil.

CONCLUSIONS

(1) Simulation modelling can be used successfully to predict water and air contents in soil for extended periods of time. Such data may be useful to define physical conditions for life in the soil and to explain biotic data and their fluctuation. In situ biological experiments may be more effective when combined with a physico-chemical characterization of the soil medium, as a function of time.

(2) "Soil Structure" is very difficult to characterize. Basic hydraulic characteristics, such as hydraulic conductivity and moisture retention, allow a dynamic characterization of structural physical and chemical properties in time when used in simulation models. Such dynamic properties are bound to be better related to biotic characteristics than the often used static soil structural properties, such as bulk density and porosity. Changes with time in soil structure at any given location (which are not considered in this study) can also be expressed in terms of changing basic hydraulic characteristics.

(3) When characterizing soil structure dynamically with modelling, it is important to express the effects of spatial variability because ranges of values obtained when expressing the variability of basic parameters for modelling may be considerable, as is demonstrated in this study.

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Modelling the geometry of worm burrow systems in relation with oxygen diffusion

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ABSTRACT

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An approximate method is described that enables the application of diffusion models to a system of worm burrows. The geometry of the real burrow system is replaced by a model system consisting of soil cylinders with different radii, each with a worm burrow along its axis. A process model, describing for instance oxygen diffusion and soil respiration, is then applied to the cylinders of the model system. Each cylinder radius represents a characteristic length scale that occurs in the system and a weight factor expresses the relative abundance of each scale. The weights are obtained from the statistical distribution of the distance from a point in the soil to the nearest burrow axis. Hence, application of the method requires distance measurements in three dimensions. In case of randomly located burrows, the distance distribution can also be obtained from measurements in a cross section of the system.

As an example, cross sections of a sulphide-containing, homogeneous clay soil are studied. The black clay has become partly oxic due to the presence of worm burrows from which oxygen has entered the soil. The oxidized soil has a light color. Digitized photographs of cross sections are analyzed with the help of an oxidation model for randomly positioned burrows. Assuming a constant distance over which oxygen has entered the soil, predictions are made about the pattern of oxidized soil visible at a cross-sectional surface. These model results are compared with the observations. The assumption of a fixed penetration distance does not explain the observed patterns. Differences in burrow age form the most likely explanation.

INTRODUCTION

In soil biology considerable attention is paid to the geometry of earthworm burrow systems (e.g. Kretzschmar, 1988; Kretzschmar and Aries, 1991; Joschko et al., 1991; Ligthart et al., 1992). The main reason for studying bur-

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row systems is their role in transport processes in the soil. In this paper, diffusion of oxygen in the soil between the worm burrows is considered. It is assumed that the concentration gradients in the burrows themselves are relatively small.

In the description of a diffusion process for a burrow system, two steps can be distinguished. In the first place, a model is required of the process taking place in the soil around individual worm burrows. Such a process model will usually consist of a differential equation describing diffusion in a soil cylinder with a worm burrow along its axis. The diffusion coefficient may depend on the water content of the soil, there may be source or sink terms like soil respiration, and the burrow surface may have special properties. The second step is the application of such a process model to a burrow system with a measured or an assumed geometry. This paper concentrates on the second step, the treatment of the geometry of the burrow system in diffusion models.

The complicated geometry of a real burrow system is replaced by a model system. The description of a diffusion process taking place in this model system is relatively simple and forms an approximation of the process in the real system. The model system consists of soil cylinders with different radii, each with a worm burrow along its axis. The relative importance of the various cylinder radii is expressed as weight factors, which are derived from the statistical distribution of the distance from a point in the soil to the nearest worm burrow. Hence, a cylinder model system can be derived from the real burrow system in an operational way and can be used to apply a variety of process models to the burrow system.

The use of weight factors for a series of cylinder radii was originally developed for aggregated soil and root systems (Rappoldt, 1990, 1992). After a brief description of the analogous method for worm burrows, it is shown how a model system can be derived from measurements made in a cross section. Then, some limitations of the method are discussed. It is not possible, for instance, to use a cylinder model system for predicting oxygen concentration patterns for a cross section of a burrow system, which could then be compared with concentration measurements in real cross sections. For an extremely simple process model, this problem has been solved by constructing on a computer a set of lines and intersecting the generated system.

The described methods are applied in analyzing a burrow system of the ragworm *Nereis diversicolor* in a young, homogeneous and sulphide-containing clay soil. From the worm burrows oxygen has entered the soil, leading to a color change due to sulphide oxidation. Photographs of cross sections have been digitized and analyzed. The non-regular geometry of the burrow system alone does not explain the oxidation patterns visible in cross-sectional surfaces. Differences in burrow age have to be taken into account.

METHODS

At first it is briefly shown how a cylinder model system can be found from the statistical distribution of the distance from a point of the soil to the nearest worm burrow. The distances should be measured in a three-dimensional picture of the burrow system. If observations are made in cross sections only, one may attempt to derive the required distance distribution from measurements in two dimensions. In case of random burrow positions this is not difficult and a few relevant formulas are given.

A cylinder model system represents only the characteristic length scales occurring in a burrow system. Since the soil cylinders of the model system have no positions in space, no meaningful cross sections exist. In order to generate pictures of cross sections, one has to assign positions and orientations to worm burrows in some kind of spatial model system. In this paper, a simple oxidation model is applied to a model system consisting of lines with random po-

TABLE 1

Description of symbols used in the text. The subscripts "v" and "s" stand for "volume" and "surface" properties, respectively

Symbol	Description	Units	Equation
A	radius of sphere containing lines	m	
d	distance vector of unit length	m	(9)
$e_{1,2}$	basis vectors of plane perpendicular to d	m	(10)
F	distribution function	—	(8)
h	position vector	m	(11)
h'	intersection of line with plane $z=0$	m	(12)
L_s	burrow density in intersecting plane	m^{-2}	
L_v	burrow length density in clod	m^{-2}	
N	number of distance and cylinder classes	—	(1)
p_i	distance probability, $i=1,\dots,N$	—	
$p_s(\theta)$	probability density of θ in cross section	—	(2)
$p_v(\theta)$	probability density of θ in clod	—	(3)
θ	polar angle	rad	
r_i	random number, uniform in $[0,1]$	—	
R_i	series of cylinder radii, $i=1,\dots,N$	m	
s_s	distance p.d.f. for cross section	m^{-1}	
s_v	distance p.d.f. for clod	m^{-1}	
t	testpoint in intersecting plane	m	
v_i	weight belonging to R_i , $i=1,\dots,N$	—	(1)
x_s	distance to nearest burrow intersection	m	
x_s^*	dimensionless value of x_s	—	
x_v	spatial distance to nearest burrow	m	
x'_v	distance to intersection of nearest burrow	m	
x_v^*	dimensionless value of x_v	—	
z	vertical coordinate	m	

sitions and non-random orientations. The results are used later in analyzing field data.

Table 1 gives a list of symbols with definitions and units.

The derivation of weight factors from the distribution of 3D distances

If the worm burrows are parallel and equally spaced (Fig. 1), a single soil cylinder forms a satisfactory model of the burrow system. The (oxygen) concentration profiles around all burrows are identical and closely resemble the concentration profile in a soil cylinder with a burrow along its axis. Hence, in case of a regular distribution of worm burrows, diffusion takes place at a single characteristic length scale only, the radius of the equivalent soil cylinder.

Clearly, real burrow systems are not regular, which implies that diffusion of solutes or gas will take place at different characteristic length scales in different parts of the burrow system. This can be described by means of soil cylinders with different radii, each with a worm burrow along its axis. To each radius R_j belongs a weight factor v_j , which expresses the relative importance of the characteristic length scale R_j in the burrow system. The model system shown in Fig. 2b represents a burrow system with random burrow positions and a density of 100 meter of burrow per m^3 of soil.

The weight factors of a model system are found from a distance distribution. For each point of the soil there is a distance to the nearest worm burrow. After determining this distance for a large number of points, a histogram can be made as in Fig. 2a. This histogram consists of the fractions p_i ($i=1,\dots,N$) of the distances found in a number of distinct size classes. These fractions may be regarded as probabilities belonging to the distance classes. Finally, the

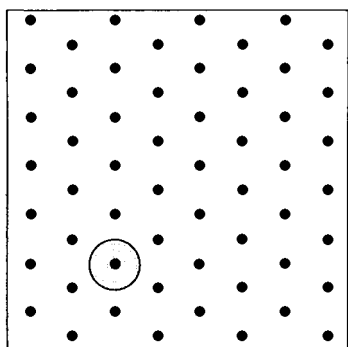


Fig. 1. A system of parallel and equidistant worm burrows can be represented by a single soil cylinder with a worm burrow along its axis.

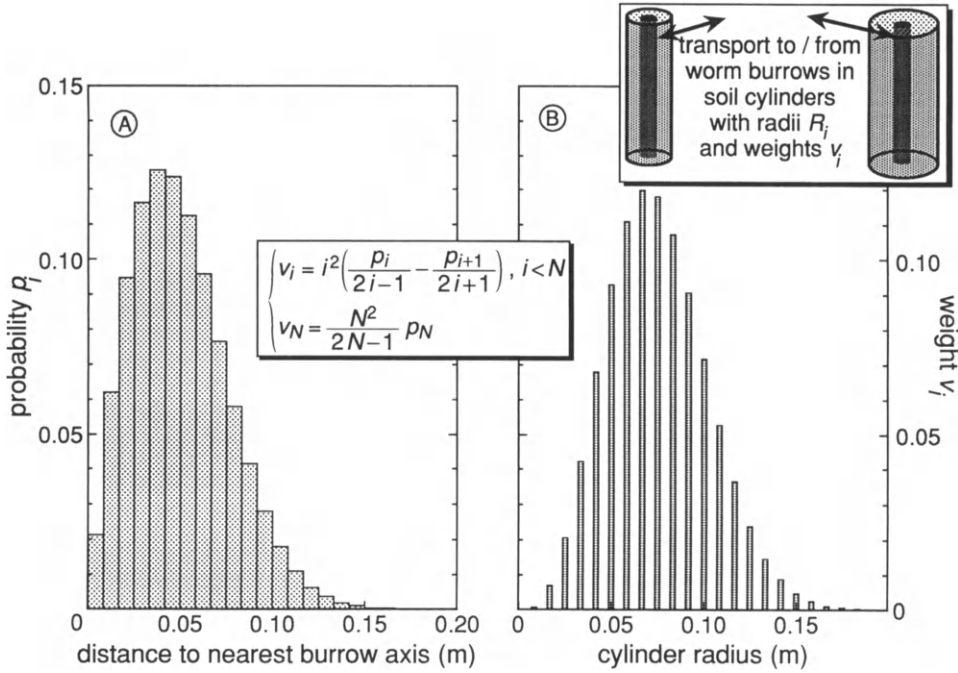


Fig. 2. In case of a non-regular distribution of worm burrows, diffusion takes place at different length scales simultaneously, which requires a model system consisting of cylinders with different radii. To each radius belongs a weight factor. (A) Distance probabilities for a system of randomly distributed worm burrows with a burrow length density of 100 m^{-2} ; (B) The weights factors derived from these distance probabilities with eq. (1).

cylinder radii R_i of the model system are set equal to the upper bounds of the distance classes and the weights are calculated with:

$$\begin{cases} v_i = i^2 \left(\frac{p_i}{2i-1} - \frac{p_{i+1}}{2i+1} \right), & i < N \\ v_N = \frac{N^2}{2N-1} p_N \end{cases} \quad (1)$$

Note that, measuring distances, the position of a worm burrow should be taken as the position of its central axis.

Equation (1) is valid only for distance classes of equal width and has been derived in Rappoldt (1990) from the requirement that the distance probabilities p_i are the same for the real burrow system and the model system. A more complete description of the mathematics underlying eq. (1) can be found in Rappoldt (1992), in which also formulas are given for unequal class widths and for continuous distance and radius variables.

The soil cylinders of a model system do not form a direct geometrical sim-

plification of a real burrow system. The cylinders do not represent individual burrows with the soil around them. They are obtained from a statistical property of the burrow system (e.g. the distance distribution) and thus represent the burrow system as a whole. The main advantage of this somewhat abstract nature of the model system is that process models describing diffusion in a single soil cylinder can be easily applied to a whole burrow system. The model results for the various cylinder radii are calculated separately and a weighted sum forms the approximate result for the real system. For instance, when for each cylinder an anoxic volume fraction is calculated with help of a model on oxygen diffusion and soil respiration, the weighted sum of these fractions is the approximate anoxic fraction of the whole soil.

The distances on which a model system is based are true spatial distances. Hence, the derivation of a model system requires a picture of the three-dimensional structure of the burrow system. It is only the *distribution* of the 3D distance that is needed, however. If the 3D distance distribution can be derived from measurements in a cross section of the burrow system, the weight factors can be obtained as well. In case of random burrow positions this is relatively easy, and the required formulas are given in the next two sections.

Density and orientation of burrows in a cross section

The burrow length per unit soil volume is written as L_v . The corresponding property of a horizontal cross section is L_s , the number of intersected burrows per unit surface area. Clearly, when all burrows are parallel and perpendicular to the intersecting plane, L_s is equal to L_v . In general, however, L_s and L_v have different values and their relation depends on the orientation of the worm burrows.

The orientation of a straight burrow can be described with a polar angle θ and an azimuthal angle φ . The polar angle is measured relative to a vertical axis. The system is assumed to possess azimuthal symmetry, which means that all values of φ between 0 and 2π are equally abundant.

The burrow length (per unit volume) with a polar angle θ in the interval $[\theta, \theta + d\theta]$ is $L_v p_v(\theta) d\theta$. Here $p_v(\theta)$ is a probability density function describing the relative abundance of the different angles θ . An analogous function $p_s(\theta)$ is defined for a cross section. It describes the orientation of intersected burrows. The relation between $p_v(\theta)$ and $p_s(\theta)$ is:

$$L_s p_s(\theta) = L_v p_v(\theta) \cos \theta \quad (2)$$

This equation expresses that the (relative) presence of burrows in an intersecting plane decreases with $\cos\theta$. The reason is that burrows with a large angle θ are rarely intersected by a horizontal plane.

From eq. (2) practical relations are derived. Values of $p_v(\theta)$ can be found from the distribution of angles θ measured at the surface by using:

$$p_v(\theta) = \frac{1}{L_v/L_s} \frac{p_s(\theta)}{\cos \theta} \quad (3)$$

Before this expression can be used, however, the ratio L_v/L_s needs to be found. As the integral of eq. (3) should be equal to unity, is:

$$\frac{L_v}{L_s} = \int_0^{\pi/2} \frac{p_s(\theta)}{\cos \theta} d\theta \quad (4)$$

This shows that, using eqs. (3) and (4), volume properties can be calculated from a measured surface density L_s and from angles measured at the surface of an intersecting plane.

For an isotropic system all orientations are equally probable. The ratio L_v/L_s is 2 and the probability density functions for the angle θ become:

$$p_s(\theta) = 2 \sin \theta \cos \theta \quad (5a)$$

$$p_v(\theta) = \sin \theta \quad (5b)$$

For these functions eqs. (3) and (4) are readily verified.

The distance probability density function in a cross section

For test points in a cross section, only surface distances x_s to the nearest burrow intersection can be measured. When the burrows are not vertical, the measured distances will be larger than the true spatial distances x_v from which a model system can be derived. It is important therefore to know how the probability density function $s_s(x_s)$, which can be measured in a plane, is related to $s_v(x_v)$.

If the burrow system consists of long straight burrows with a random position, the relation between $s_s(x_s)$ and $s_v(x_v)$ is very simple. The functions have exactly the same form:

$$s_v(x_v) = 2\pi L_v x_v \exp(-\pi L_v x_v^2) \quad (6)$$

$$s_s(x_s) = 2\pi L_s x_s \exp(-\pi L_s x_s^2) \quad (7)$$

Equation (6) was derived by Ogston (1958) for an isotropic system. In fact, he derived an expression for rods with a finite length. Here, (infinitely) long lines are considered and Ogston's expression has been simplified. For an isotropic system the burrow density L_v , which is the only parameter in eq. (6), is twice the surface density L_s . As mentioned also by Barley (1970), Ogston's result remains valid for an anisotropic angular distribution. In that case, the value of L_v can be determined from a measured value of L_s and the ratio L_v/L_s , calculated with eq. (4) from measured polar angles. The randomness of the burrow spacing can be verified by comparing a measured function $s_s(x_s)$

with eq. (7). Hence, in case of randomly located, long burrows, the probability density function $s_v(x_v)$ can be derived from measurements in a cross section.

From $s_v(x_v)$ a cylinder model system can be derived with an expression given in Rappoldt (1992). The weight density or volume fraction density $v(R)$ belonging to a cylinder with radius R becomes:

$$v(R) = 2\pi^2 L_v^2 R^3 \exp(-\pi L_v R^2)$$

Alternatively, one may calculate from $s_v(x_v)$ a number of discrete probabilities belonging to a number of distance intervals. Then, eq. (1) can be used to calculate a series of discrete weights.

Limitations of a cylinder model system

When (oxygen) concentration patterns around worm burrows develop in time, differences in burrow age have to be taken into account. A single, old worm burrow, for instance, may have led to the oxidation of a relatively wide zone around it, containing even newer burrows. Such a situation cannot be described with a set of separate cylinders.

A second limitation is that properties which are defined for cross-sectional surfaces only, cannot be generated with a cylinder model system. For instance, the oxygen concentration can be measured in a cross section as function of the distance x_s to the nearest burrow intersection. Distances x_s , however, do not exist in the model system and predictions in terms of x_s cannot be made. In Fig. 3 this is demonstrated by means of a schematic drawing.

To a certain point of a cross section belongs a distance x_v to the nearest burrow. In the cross-sectional plane two more distances can be distinguished. The first is the distance x'_v to the intersection of the nearest burrow. This distance is related to x_v and the burrow orientation. The second distance is

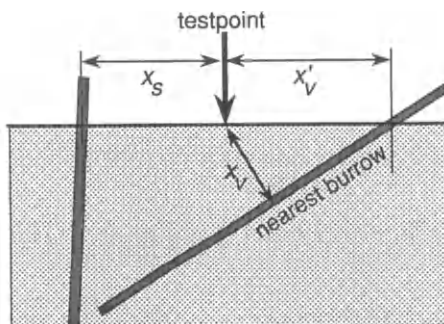


Fig. 3. A test point in an intersecting plane has a spatial distance x_v to the nearest worm burrow, a surface distance x'_v to the intersection of the nearest burrow and a surface distance x_s to the nearest burrow intersection.

x_s , the distance to the nearest burrow intersection, which is not necessarily the intersection of the nearest burrow. Hence, the distances x'_v and x_s need not to be equal and their distributions will differ.

After assigning orientations to the cylinders of the model system, according to a (measured) angle distribution, the model system could be intersected and, for each point of the system, a distance x'_v could be calculated. Surface distances x_s would still not exist, however. In short, a cylinder model system can be used to apply process models to the burrow system and to predict average concentrations or concentration distributions as function of x_v . It cannot be used to predict surface properties measured as function of the distance x_s . This is even the case for random burrow positions, when, as shown in the previous section, the distributions of x_s and x_v are closely related.

A way of solving this problem, at least for random burrow positions, is to generate a model system consisting of straight lines with random positions. The lines represent axes of worm burrows and the orientation of the lines may be non-random. Clearly, for the points of a cross section of such a system, all three distance types, x_v , x'_v and x_s , exist. The consequence of introducing burrow positions and orientations, however, is that it becomes difficult to apply a process model. One cannot make use anymore of the simple cylindrical geometry. The calculations below are carried out for an extremely simple process only. It is assumed that all worm burrows are equally old and that the homogeneous soil around them has slowly oxidized. The soil has become oxic up to a certain threshold distance from the burrows. Such a process model does not require any calculations on the diffusion process itself, since the status of a point, either oxic or anoxic, is determined by the distance x_v belonging to it. Then, for each point of a cross section, a surface distance x_s and a volume distance x_v can be found. By choosing many points in a cross section, the probability of finding an oxic point can be calculated as function of x_s .

As the generation procedure for a set of lines with a random position is not entirely trivial, it is described below in some detail.

Generation of a spatial model system

A set of straight lines is generated within a sphere with radius A and with its centre at the origin. Each line is specified by a position and an orientation. The orientation is given as a vector $\mathbf{d}$ of unit length, pointing in some direction. A direction vector $\mathbf{d}$ is determined by two angles, a polar angle θ and an azimuth angle φ . The position vector $\mathbf{h}$ points to an arbitrary point of the line. Once the line direction is known, there are only two degrees of freedom left for its position in space. A vector $\mathbf{d}$ and a vector $\mathbf{h}$ can therefore be generated from four random numbers. The generation of pairs $(\mathbf{h}, \mathbf{d})$ can be continued until a required number of lines or a required line density is reached.

The used random numbers should be uniformly distributed in the interval

$[0,1]$ and may be calculated by means of a pseudo random number generator (e.g. Bratley et al., 1987). The four numbers required for a single pair $(\mathbf{h}, \mathbf{d})$ are written as r_1, r_2, r_3 and r_4 .

If there is azimuthal symmetry, a value for the azimuth angle φ is found from the first random number in a trivial way:

$$\varphi = 2\pi r_1$$

In case of isotropy, the probability density of the polar angle θ is given above by eq. (5b). A value of θ distributed according to that function is calculated with help of the cumulative distribution function $F(\theta)$ which is:

$$F(\theta) \equiv \int_0^\theta p_v(\theta) d\theta = \int_0^\theta \sin \theta d\theta = 1 - \cos \theta \quad (8)$$

A value of θ is found then by inversion (Bratley et al., 1987) which results in:

$$\theta = F^{-1}(r_2) = \arccos(1 - r_2)$$

In case of anisotropy, a value of θ should be generated which is distributed according to any other function $p_v(\theta)$, for instance by using a series of measured probabilities.

After generating the angles φ and θ , the direction vector $\mathbf{d}$ in cartesian coordinates becomes:

$$\mathbf{d} = \begin{pmatrix} \sin \theta \cos \varphi \\ \sin \theta \sin \varphi \\ \cos \theta \end{pmatrix} \quad (9)$$

The positioning procedure should guarantee that lines with different orientations are distributed over the sphere in the same way. A position vector $\mathbf{h}$ is randomly chosen in a square perpendicular to the generated direction $\mathbf{d}$. The area of that square is A^2 , independently of $\mathbf{d}$. Hence, the density resulting from N lines with a certain orientation will be equal to the density resulting from N lines with a different orientation.

The plane through the origin and perpendicular to $\mathbf{d}$ has an orthonormal basis formed by the two vectors $\mathbf{e}_1$ and $\mathbf{e}_2$ given by:

$$\mathbf{e}_1 = \begin{pmatrix} \cos \varphi \cos \theta \\ \sin \varphi \cos \theta \\ -\sin \theta \end{pmatrix}, \quad \mathbf{e}_2 = \begin{pmatrix} -\sin \varphi \\ \cos \varphi \\ 0 \end{pmatrix} \quad (10)$$

The third and fourth random number are now used to get two coordinates of $\mathbf{h}$ with respect to this basis. The coordinates both lie in the interval $[-A, +A]$. $\mathbf{h}$ is formed then as a linear combination of $\mathbf{e}_1$ and $\mathbf{e}_2$ according to:

$$\mathbf{h} = (1 - 2r_3)A\mathbf{e}_1 + (1 - 2r_4)A\mathbf{e}_2 \quad (11)$$

The position vector $\mathbf{h}$ may be rejected when it is not inside the sphere. In that case new numbers r_3 and r_4 have to be generated. Rejection is not necessary, however. The intersection of a generated line with the “horizontal” plane $z=0$ is written as the vector $\mathbf{h}'$. When $\mathbf{h}'$ exists it is found as:

$$\mathbf{h}' = \mathbf{h} + [(1 - 2r_3)A \tan \theta] \mathbf{d} \quad (12)$$

The calculation of the spatial distance from a test point $\mathbf{t}$ to the line given by $(\mathbf{d}, \mathbf{h})$ is simplified by the use of vector cross products. When the angle between two vectors $\mathbf{a}$ and $\mathbf{b}$ is α , the length of the cross product is (e.g. Lang, 1970):

$$\|\mathbf{a} \times \mathbf{b}\| = \|\mathbf{a}\| \|\mathbf{b}\| |\sin \alpha|$$

Using this property of vector cross products and $\|\mathbf{d}\| = 1$ the distance from a line to a test point $\mathbf{t}$ becomes $\|(\mathbf{t} - \mathbf{h}) \times \mathbf{d}\|$. If the test point lies in the plane $z=0$, the distance to a line intersection $\mathbf{h}'$ becomes $\|\mathbf{t} - \mathbf{h}'\|$.

After generating N vector pairs $(\mathbf{d}_i, \mathbf{h}_i)$ with $i = 1 \dots N$, test points $\mathbf{t}$ are chosen in the plane $z=0$. The distance x_v from a test point $\mathbf{t}$ to the nearest line is:

$$x_v = \min_i \|(\mathbf{t} - \mathbf{h}_i) \times \mathbf{d}_i\|$$

and the distance from the same test point to the nearest line intersection is found by calculating for all lines the intersection points $\mathbf{h}'$. Then:

$$x_s = \min_i \|\mathbf{t} - \mathbf{h}'_i\|$$

Surface patterns

With the method described above, an isotropic system was generated and a grid of test points was chosen in the intersecting plane $z=0$. Figure 4 shows, as a function of x_s , the fractions of points with a distance x_v in a number of intervals. The distances have been made dimensionless according to:

$$x_s^* = x_s \sqrt{\pi L_s} \quad (13a)$$

$$x_v^* = x_v \sqrt{\pi L_s} \quad (13b)$$

Note that the surface density L_s has been used to scale both the surface and the spatial distances. The graph demonstrates that, especially for a large surface distance, very different spatial distances occur. After choosing a certain threshold distance x_v , above which the soil is anoxic, a curve as in Fig. 4 becomes the probability of finding oxic soil as function of x_s .

Figure 5 has been calculated for the measured distribution of the polar angle θ , which expresses a preference for vertical burrows (see Fig. 7 below). The correspondence between surface and spatial distances is better than in

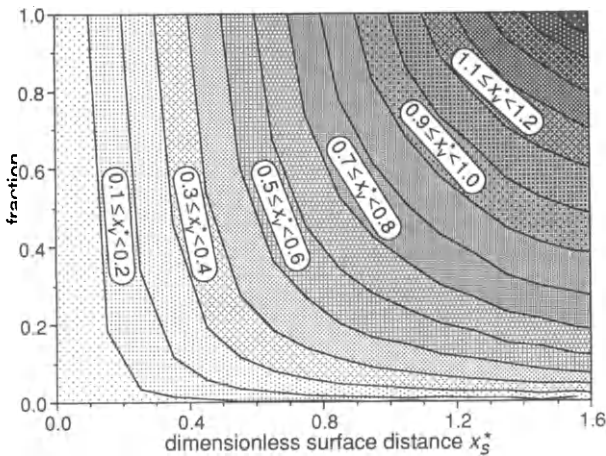


Fig. 4. Test points with a certain dimensionless surface distance x_s^* ($=x_s \sqrt{\pi L_s}$, horizontal axis) lie at different spatial distances x_v^* ($=x_v \sqrt{\pi L_s}$) from the nearest burrow. Calculations have been made for an isotropic system of randomly positioned straight lines.

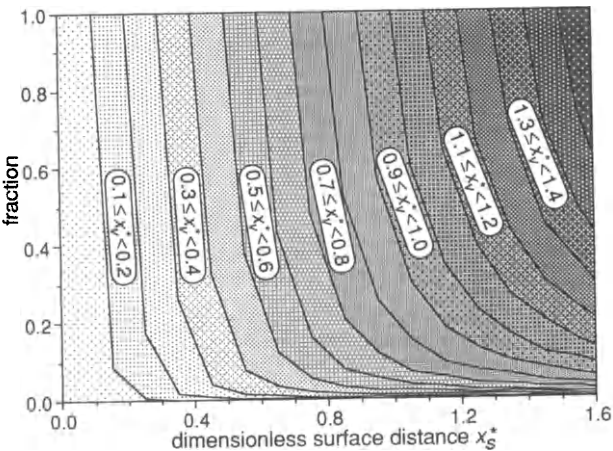


Fig. 5. The same graph as Fig. 4, now based on the measured distribution of the polar angle θ (see Fig. 7). The preference for a vertical burrow orientation leads to a better correspondence between spatial and surface distances than in Fig. 4.

Fig. 4. In case of vertical burrows the spatial distances would exactly correspond to surface distances and all curves would be vertical between 0 and 1.

SOME EXPERIMENTAL RESULTS

A clay soil has been studied in which worm burrows occur, which have a profound influence on soil aeration. The soil far from the burrows contains sulphide and is black. Close to the burrows the sulphide has oxidized and the

soil has a much lighter color. Except the worm burrows, the soil does not show any structure on a relevant scale and is assumed to be homogeneous. Patterns of oxidized soil observed in a cross section are compared with results of a simple model of soil oxidation. In accordance with the calculations summarized in Figs. 4 and 5, it is assumed that all burrows are equally old and that the soil up to a certain threshold distance from the burrows is oxidized. Predictions based on these assumptions appear to disagree with the observed distribution of oxic soil.

Description of the soil

The soil studied is situated on the island Schiermonnikoog in the Dutch part of the Wadden Sea. Between the dike along the southern part of the island and the tidal flats lies a strip of salt marsh. On this salt marsh small depressions occur with a typical size of 20×40 m. They are a few years old and have been created as ~ 1 m deep clay excavations during a recent dike reconstruction. During periods of inundation a new layer of clay has been deposited on the bottom. The clay is black, due to the sulphide formed under anoxic conditions.

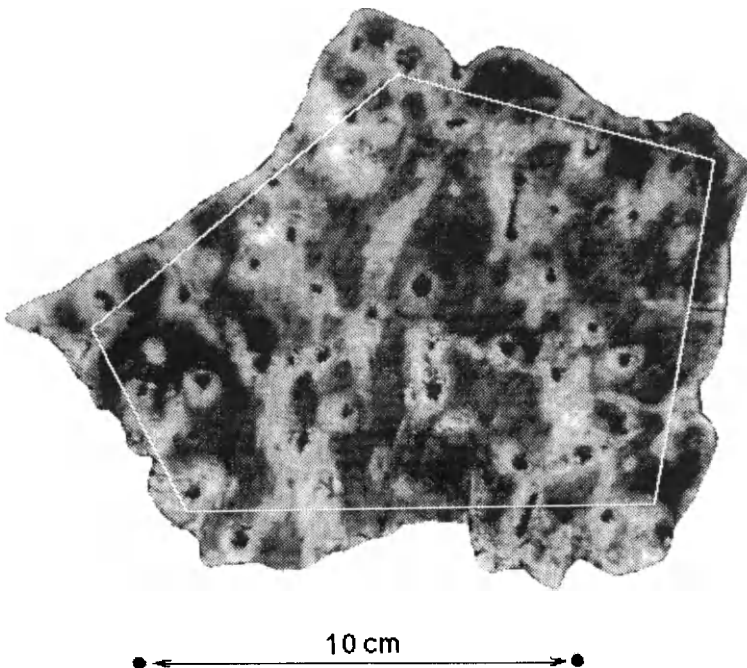


Fig. 6. Horizontal cross section of a clay prism with burrows of the ragworm *Nereis diversicolor*. The dark soil contains sulphide and in the light zones the sulphide has oxidized.

In spring and summer prolonged periods of drought occur. Water evaporates from the excavations, the clay soil becomes dry and ~ 20 cm deep cracks have developed. The cracks form the pattern which is characteristic for a cracked clay soil. In autumn and winter the evaporation is lower and the depressions are usually inundated. The water probably has a strongly varying salinity and comes in during storm tides and rainfall.

The soil in the depressions is bare. No pioneer plants in the region have been able to withstand the harsh conditions. The only larger organism present is the ragworm *Nereis diversicolor* living in burrows inside the clay prisms. These worms are able to survive dry periods by hiding up to 30 cm below the surface where the soil remains wet throughout the year.

A horizontal cross section of a clay prism shows a number of worm burrows (Fig. 6). Around the burrows there are lighter zones. Apparently, oxygen has entered the soil from the worm burrows, leading to the oxidation of sulphide and the local disappearance of the black color.

Experimental procedure

Cross sections of clay prisms were made at a depth of about 6 cm using a steel wire with a diameter of 0.2 mm. The black and white photographs taken of the surfaces have been digitized. A digitized picture is a file consisting of 262,144 grey values organized as 512 lines of 512 pixels. Each grey value is a number between 0 and 255 (one byte). The sulphide-containing, black parts of the surface appear in the file as pixels with a low grey value. Also the worm burrows themselves are dark spots. Some noise was removed from the image by applying a 5×5 median filter (e.g. Methir, 1975). The coordinates of the burrows in the picture have been determined by hand. Two black dots at a distance of 10 cm provide a reference length on all photographs.

The orientation of a worm burrow was determined in the field by putting in a thin stick and measuring the angle between the stick and the (horizontal) cross-sectional surface. The result is easily converted to the angle θ between the burrow direction and a vertical line.

With help of the image files and burrow positions the relation between the "color" of test points and their distance x_s to the nearest burrow intersection can be quantified.

Orientation and distribution of burrows

482 measured polar angles θ were classified using nine angle classes of equal width. For each class a probability $p_{i,s}$ has been calculated as the fraction of angles in that class ($i=1\dots 9$). Figure 7 shows the result as a histogram of probability densities (probabilities divided by the constant class width). The curve in Fig. 7 is a graph of the function $p_s(\theta)$ for an isotropic system (eq.

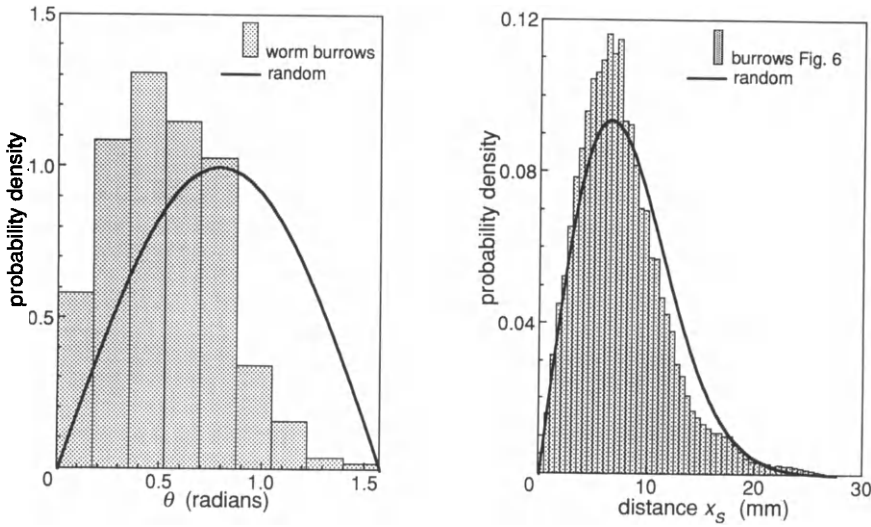


Fig. 7. Frequency distribution of the polar angle θ between a vertical line and a worm burrow appearing at an intersecting plane. The curve describes an isotropic system (eq. 5a). The histogram is based on 482 measured angles and reflects the preference of the worms for a vertical burrow orientation.

Fig. 8. The distribution of the surface distance x_s between a test point and the nearest burrow intersection. The histogram gives probability densities, measured for test points in the polygonal test area drawn in Fig. 6. The curve describes a random burrow position (eq. 7 with $L_s = 0.38 \text{ cm}^{-2}$).

5a). The difference between the histogram and the curve reflects the preference of the worms for vertical burrows.

From the surface probabilities $p_{i,s}$ the ratio L_v/L_s can be estimated as (cf. eq. 4):

$$\frac{L_v}{L_s} = \sum_{i=1}^9 \frac{p_{i,s}}{\cos \theta_i}$$

The midpoints of the classes have been used as angles θ_i . The result is a ratio 1.3 ± 0.1 . This value is sensitive to the occurrence of angles close to 90° and the given inaccuracy of 0.1 is an estimate. Using this ratio L_v/L_s volume probabilities $p_{i,v}$ can be found from (cf. eq. 3):

$$p_{i,v}(\theta) = \frac{1}{L_v/L_s} \left\{ \frac{p_{i,s}(\theta)}{\cos \theta_i} \right\}$$

These probabilities have to be used to generate the above described spatial model of the burrow system.

A polygon drawn in the digitized image in Fig. 6 gives the area in which test points have been chosen. The burrow density L_s in the test area is 0.38 cm^{-2} .

Using the average value for L_v/L_s above the estimated volume density L_v becomes 0.50 cm^{-2} .

Using the positions of the worm burrows, distance probabilities have been calculated by determining for each pixel in the test area the distance to the nearest burrow centre. The histogram in Fig. 8 shows the result. The curve refers to random burrow positions (eq. 7 with $L_s = 0.38 \text{ cm}^{-2}$). The difference in shape between the histogram and the curve reflects a tendency towards a regular distribution of burrows. The statistical significance of this tendency has not been verified, but the same result has been found in other photographs. The deviation from random burrow positions has been neglected in the analysis of the oxidation pattern.

Sulphide oxidation

At first a criterion has to be found for classifying pixels of the image as either “dark” or “light” (oxidized). For the image in Fig. 6 the criterion is derived from the histogram of grey value frequencies in Fig. 9. The histogram shows a clear peak for low grey values. That peak represents the dark, sulphide-containing parts of the surface. The grey value 75 at the right edge of the peak is chosen as the threshold value. Pixels with a lower grey value are classified as dark and pixels with a higher grey value are classified as light. The image resulting from this choice is given in Fig. 10.

Calculating the distances x_s for the pixels in the test area, also the dark or light nature of the pixels has been scored. That results in a fraction of light pixels as function of the distance x_s . Figure 11a shows a graph of the result. Many points with a distance below 2.5 mm are dark, simply because they are

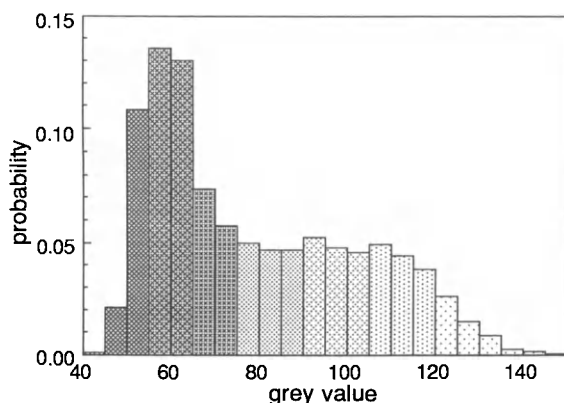


Fig. 9. The grey value distribution of the pixels in the polygonal test area drawn in Fig. 6. The distinction between dark, sulphide-containing soil and oxidized soil lies at a grey value of about 75.

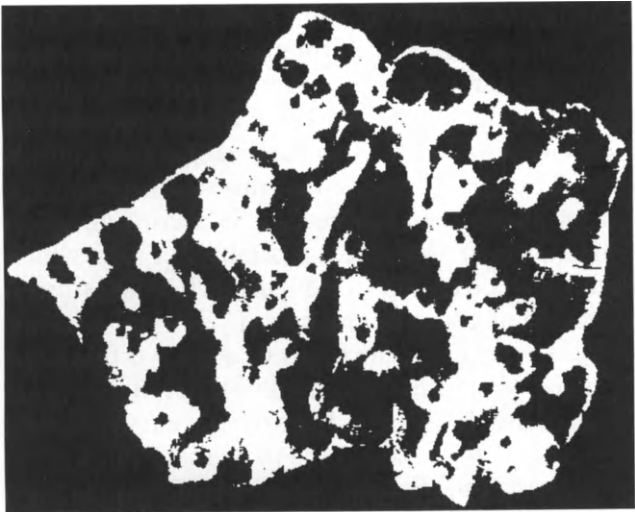


Fig. 10. The clay prism of Fig. 6 with grey values from 0 to 75 classified as anoxic (black) and the other pixels as oxic (white). Note that the burrows themselves are also black.

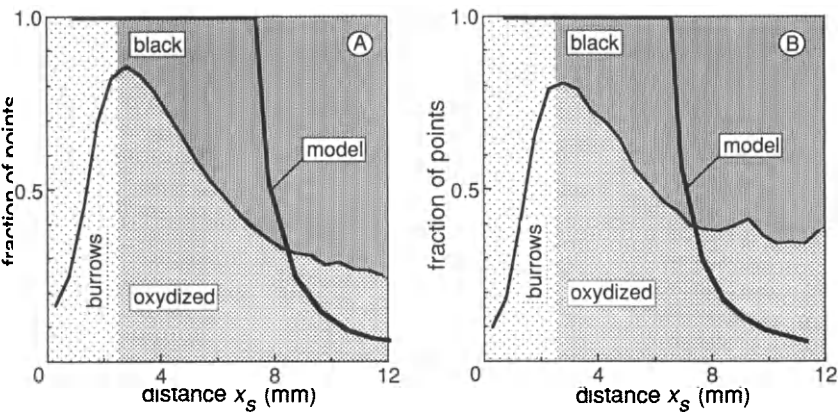


Fig. 11. Test points at a distance x_s from the nearest burrow intersection have been classified as either dark or light (oxidized). Many pixels at distances below 2.5 mm are part of the (dark) burrows themselves. Above 2.5 mm, the fraction oxidized soil decreases with distance. The fat, steeply decreasing curve is the model result for randomly positioned straight lines (Fig. 4). (A) Result for the clay prism in Fig. 6; (B) The same photograph of another clay prism.

part of a worm burrow. Burrows with a radius above 2.5 mm are rare (the burrow radius measured in the field is 1.5 ± 0.3 mm) and from that distance on, the fraction of light, oxidized soil decreases and the fraction of dark, sulphide-containing soil increases.

By combining the histogram in Fig. 8 with the light fraction in Fig. 11a, the light surface fraction can be calculated. It appears to be 47%. The surface

fraction occupied by the burrows is about 2.5%, leading to a total oxic surface fraction of 50%. Since, for large samples, surface fractions are equal to volume fractions the estimated oxic volume fraction amounts also 50%.

This last number can be used to calculate a theoretical curve based on the assumption of a certain threshold distance above which all pixels are dark. For an oxic volume fraction of 0.50, eq. (6) leads to a threshold distance of 7.6 mm corresponding to a dimensionless distance of 0.83 according to eq. (13b). For a dimensionless distance of 0.8 a curve is available in Fig. 5, which has been adapted to the millimeter scale of Fig. 11a. Figure 11b shows similar graphs derived for the cross section of a second clay prism.

The theoretical curves in Fig. 11 represent the effect of a non-vertical burrow orientation. When all burrows would be vertical, the theoretical curves would be vertical lines at the threshold distance. Instead, the curves have a tail, which is caused by the fact that surface distances in a cross section may be larger than true spatial distances x_v to the nearest burrow. The measured curves in Fig. 11, however, show a still slower decrease of the oxidized fraction with distance. The difference with the steep model curves seems large enough to conclude that a simple threshold distance cannot explain the observed oxidation patterns.

The slow observed decrease of the oxidized fraction with distance can be explained by differences in threshold distance between worm burrows. These differences may originate from differences in burrow age or in the local sulphide content of the soil. By assigning different threshold distances to the lines of the model system underlying Fig. 5, a slowly decreasing fraction could be easily described. Since threshold distances have not been measured for a sufficient number of burrows, however, such calculations have not been carried out.

A second factor leading to variation in threshold distance is that the oxidation front around a few neighboring burrows does not correspond precisely with a single threshold distance. This effect alone, however, cannot account for the large differences found.

CONCLUSIONS

Approximate calculations on diffusion in the soil between worm burrows can be carried out for a simple cylinder model system instead of for the complicated geometry of the real burrow system. The cylinder system is based on the distribution of the spatial distance from a point of the soil to the nearest burrow axis. An important advantage of this method is that a diffusion process needs to be described only for a single soil cylinder with a worm burrow along its axis. Then, the process model can be applied to a cylinder system, which represents the different length scales occurring in the burrow system. For each cylinder radius, a weight factor specifies the relative importance of

the corresponding length scale. Overall results of a process model are then calculated as weighted sums of the results for all cylinders. The weights are derived from the requirement that the model system has the same distance distribution as the burrow system.

In a cylinder model system, the worm burrows do not possess an orientation or a position. Only the different length scales of the system are represented. If this is not sufficient, more detailed geometrical models have to be made in which the burrows are represented by straight lines, for instance. This greater detail leads to problems, however, when a diffusion process needs to be described. Usually numerical methods will have to be used.

A cylinder model system works best for describing a steady-state diffusion process. When oxygen concentrations around each burrow depend on the history of the burrow, differences in burrow age make it impossible to use a model system based on length scales only. For a burrow system studied in the field, a model based on equal burrow age could be shown to be inconsistent with observed cross-sectional oxidation patterns.

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Relationships amongst organic matter content, heavy metal concentrations, earthworm activity, and soil microfabric on a sewage sludge disposal site

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ABSTRACT

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From 1973 to 1980, sewage sludge precipitated by three chemical treatment processes (aluminum sulphate, ferric chloride and calcium hydroxide) was applied to replicated plots on a silt loam soil at four different rates. The sludge was contaminated by heavy metals associated with industrial activity in the metropolitan areas from where it was collected. The grass on the plots has been mown regularly from 1973 onwards. Soil and earthworm populations were subjected to analysis in 1989 and 1990.

Soil pits were dug and sampled to a depth of 50 cm in four plots receiving the highest rates of the three different types of sewage treatment processes (and a control plot treated through the same period with ammonium nitrate) to determine heavy metal contents and soil organic matter distributions with depth.

The earthworm fauna of the plots was almost entirely represented by populations of *Lumbricus terrestris* L. that were analyzed for abundance and biomass following collection using the formalin expulsion method. Earthworm biomass was higher in sludge treatments than in control plots. Cadmium concentrations in earthworm tissues were correlated with soil Cd concentrations. Only worms from Al-sludge treatments had elevated tissue concentrations of Cd. Cadmium distributions in sludge-treated soils were correlated with organic matter distributions in the soil profile (for individual profiles). Intact blocks of soil were removed from soil pits for chemical analysis of the soil microfabric by PIXE (proton induced X-ray emission) and electron micro-probe (EMP). Concentrations of several trace metals on the inner walls of the earthworm channels correlated with those added to the soil via the sludge. Water flow through earthworm burrows lined with fecal material, which had higher metal concentrations than the adjacent soil matrix, could move soluble forms of the elements, thus accelerating leaching of metals into the aquifer from surface-applied sludge.

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Mapping of elemental distributions in pedo-features of biological origin will be a powerful technique for determining contributions of soil fauna and microflora to biogeochemical processes.

INTRODUCTION

Disposal of sewage sludge from waste treatment plants is of considerable environmental concern. During the 1970's, there was wide interest in spreading sewage sludge from municipal sewage treatment plants on agricultural and forested lands as a method for its disposal, and as an energy and nutrient subsidy. However, subsequent studies revealed that heavy metals from land-applied sludge could pollute soil, and taint crop plants (Mitchell et al., 1978; Carter et al., 1983; Diercxsens et al., 1985; Levine et al., 1989).

Interest in soil-Cd distributions occurs in urban environments because long abandoned waste dumps and factory sites that may have contained or used heavy metals have been converted to other uses such as parks or residential developments, that put current users and residents of these sites at risk of exposure to toxic heavy metals. A comprehensive study of a site with known inputs of heavy metal contaminants applied under controlled field conditions would allow measurement of some of the biotic and abiotic parameters that govern the distribution of heavy metals in soil.

A site that carefully accounted for heavy metal inputs exists at the University of Guelph which established a set of plots at its Elora Experimental Farm in 1973 to monitor the effects of land disposal of sewage sludge derived from different chemical precipitation processes (Soon et al., 1981). Sludge treatments were applied continuously until 1980, but our characterization study began in 1989 on the site to measure heavy metal content in soil and earthworms.

METHODS

Plot design

Sewage sludge from three different sources had been applied annually to 48 plots of 3.6 m $\times$ 2.6 m with no headland from 1973 to 1980, (Table 1). Plots were seeded with brome grass (*Bromus inermis*), and mown twice annually. Control plots received no sludge, but were treated with 0, 100, 200, or 400 kg N/ha of ammonium nitrate annually (Table 2). Sewage sludge was obtained from three different sources, each with different chemical precipitation processes, namely, calcium hydroxide, aluminum sulphate, and ferric chloride. Rates of sludge application were controlled to deliver 200, 400, 800 and 1600 kg equivalent of total N ha⁻¹ yr⁻¹. All treatments were repeated in three fully

TABLE 1

Sludge plot inputs for constituents added to bromegrass plots by annual application of sludge from 1973 to 1980 (assuming an N-application rate of $200 \text{ kg ha}^{-1} \text{ yr}^{-1}$; table adapted from Soon et al., 1981)

Constituent	Ca-sludge ^b (kg/ha) ^a	Al-sludge (kg/ha) ^a	Fe-sludge (kg/ha) ^a
Dry matter	58,900	38,200	34,500
Total N	1000	1600	1600
NH ₄ -N ^c	225	541	521
P	1240	1390	1470
Ca	10,020	2100	1270
Al	377	1260	423
Fe	1560	994	2090
Cd	< 1	3	1
Cr	41	138	22
Cu	21	63	49
Ni	78	5	1
Pb	16	42	57
Zn	94	385	84

^aFor plots receiving 1600 kg N/ha, for example, tabulated values would be multiplied by 8.

^bNo Ca-sludge was added from 1978 to 1980.

^cSoluble/exchangeable.

randomized blocks for a total of $3 \times (12 + 4) = 48$ plots. Sludge applications were suspended for the Ca-sludge plots from 1978 to 1980 (Soon et al., 1981).

Soil sampling and characterization

Soil pits were dug in one plot of one block of the highest application of N (100, 216, 316 and 416 plots, cf. Table 2) in 1989 to obtain soil samples to a depth of 50 cm. Particle size distribution, organic matter content, and heavy metal residues were determined on these samples. Intact blocks of soil $\sim 7.5 \times 9$ cm and 5 cm thick were collected from the soil pit. These blocks were impregnated with resin, hardened, and cut and polished for subsequent PIXE (proton induced X-ray emission at the University of Guelph) and EMP (electron microprobe at the University of Western Ontario) analysis.

SEM-EDX, PIXE and EMP data

Thin sections were prepared to represent earthworm channels, and made to an approximate thickness of $50 \mu\text{m}$ to ensure that the proton beam (PIXE) did not reach the glass slide. SEM-EDX was used as a quick check for trace metal concentrations on the wall linings of earthworm burrows; trace metals concentrations were found to be below the detection limits of SEM-EDX. The

TABLE 2

L. terrestris abundance and biomass for 3 sludge treatments with differing fertilizer treatment rates at Elora, Ontario, May 14, 1990

Treatment rate	Trtmt. No.	Mean ^a numbers/m ²	Mean total worm biomass (fwg ^b /m ²)	Mean individual worm biomass (fwg ^b /worm)
No sludge or N	100	43.5a (23.2a)	17.8a (35.9a)	0.51a (1.55a)
No sludge+100 kg N	101	53.7a	18.2a	0.41a
No sludge+200 kg N	102	49.1a	26.8abc	0.56a
No sludge+400 kg N	104	40.7a	20.8ab	0.53a
Ca-sludge (=200 kg N)	202	73.2ab	43.6bc	0.59a
Ca-sludge (=400 kg N)	204	52.8a	21.7ab	0.41a
Ca-sludge (=800 kg N)	208	48.2a	37.2abc	0.83b
Ca-sludge (=1600 kg N)	216	46.3a (36.1a)	13.5a (119.8c)	0.26c (3.32b)
Al-sludge (=200 kg N)	302	65.7ab	50.5c	0.83b
Al-sludge (=400 kg N)	304	44.4a	29.5abc	0.61a
Al-sludge (=800 kg N)	308	50.0a	31.3abc	0.63a
Al-sludge (=1600 kg N)	316	95.4b (49.1b)	35.0abc (80.4b)	0.37a (1.64a)
Fe-sludge (=200 kg N)	402	71.3ab	21.2ab	0.34a
Fe-sludge (=400 kg N)	404	46.3a	28.1abc	0.72a
Fe-sludge (=800 kg N)	408	59.3ab	29.8abc	0.47a
Fe-sludge (=1600 kg N)	416	65.7ab (43.5a)	19.8ab (73.9b)	0.26c (1.70a)

Data from smaller 1989 sampling are given in parentheses.

^aMeans are based on three samples. Values followed by same letter within a column are not significantly different ($P < 0.05$) by Duncan's multiple range test (1955). Analysis of variance for worm numbers: [1989] $F = 3.49$, $df = 3, 8$; $P = 0.07$ (ns); for worm weights: $F = 18.09$; $df = 3, 8$; $P = 0.0006$ (highly significant); [1990] for worm numbers: $F = 1.50$; $df = 15, 32$; $P = 0.16$ (ns); for worm weights: $F = 1.96$; $df = 15, 32$; $P = 0.053$ (almost significant).

^bfwg = fresh weight in grams.

SEM was then used to obtain backscatter images on which plasmic areas are easily differentiated from mineral grains. These images were used to locate areas for elemental analysis by PIXE and EMP.

Elemental contents were collected from the samples in two modes on the University of Guelph Micro-PIXE facility (Perujo, 1988; Campbell et al., 1990; Campbell et al., 1991) as a rastered beam producing two-dimensional imaging ($400 \times 400 \mu\text{m}$ areas), and as a beam spot of $5 \mu\text{m}$. The software package used for fitting PIXE spectra and calculating elemental concentrations is reported by Maxwell et al. (1989).

The EMP can display a backscatter image, therefore locating plasmic areas more easily than within the PIXE analysis system. Up to twelve elements were measured simultaneously by EMP with a beam diameter down to $5 \mu\text{m}$.

Earthworm sampling

Three samples of earthworms were collected from one 0.2×0.2 m square quadrat in each of the 100, 216, 316 and 416 labelled plots (total twelve) on October 14, 1989 using a modified formalin expellant method (Raw, 1959; Tomlin and Gore, 1974). Earthworms were returned to the laboratory for counting, weighing and identification. Gut contents were cleared from the worms by placing them on moist paper for 72 hours in closed containers with no food. The cleaned worms were freeze dried and the heavy metal content of their tissues determined.

Earthworms were sampled again May 14, 1990 from all 48 plots to determine abundances, biomass and heavy metal residues in their tissues. Statistical comparisons amongst treatments were performed using SPSS/PC+ (Norusis, 1988).

Heavy metal residue analyses of earthworms and soil

Freeze-dried earthworms were dissected to remove soil particles contained within the gut, because soil frequently contains naturally occurring heavy metal residues in sufficient quantity to confound earthworm tissue analysis (Andersen, 1979). Earthworms and soil samples from the different treatments were ashed at 525°C for 24 h and 100 mg aliquots of ash were placed in containers with 1.5 ml aqua regia, 2.5 ml HF, and steam heated for 2 h; 25 ml boric acid and water sufficient to give a final volume of 50 ml was added to the solution. The processed material was submitted to atomic absorption spectrophotometry (AAS) to measure heavy metal content of soil and earthworm tissue. Cd residues in soil and earthworm tissues were subjected to correlation and regression analysis using SPSS/PC+ (Norusis, 1988).

RESULTS

Soil characterization

The soil profile is classified as a Eutrochrept in the "Soil Taxonomy" (Eutric Cambisol in the "FAO-UNESCO" or World System), and a Gleyed Orthic Melanic Brunisol (Canadian System of Soil Classification). The landform is a flat plain at the toe of a drumlin with 0% slope. The parent material is silt loam alluvium.

The largest differences in inputs amongst control and sludge-treated plots were in the large amounts of organic matter applied as sludge (Table 1). The distribution of organic matter (OM) in soil horizons to a depth of 22 cm is given in Table 3; the results of higher OM inputs for the Ca-sludge plot can still be clearly detected in 1989 from the higher OM fraction down to the 15 cm horizon. Most of the organic matter has been retained in the upper 7 cm in all sludge treatments. The Fe-sludge treatments received proportionately ~10% less dry matter (DM) than the Al-sludge plots, but by 1989, the Fe-sludge plots had a larger fraction of OM (~50% more) in the upper 7 cm horizon than the Al-sludge plots.

There were significant amounts of heavy metals (Cd, Cr, Pb and Zn) added to the soil via the sludge treatments; the Al-precipitated sludge had noticeably higher levels of these metals than the other two sludges (Table 1). The Ca-sludge contributed five to eight times more calcium to the soil than Al- or Fe-sludges. The fecal pellets and burrow linings are highly enriched with Ca as compared to the soil matrix (Tables 6 to 9).

TABLE 3

Organic matter distributions in soil layers from the surface to 22 cm in the three sludge and a fertilizer (ammonium nitrate) treatments at Elora, Ontario in 1989

Depth (cm)	% Organic matter in indicated fertilizer and sludge treatments			
	Amm. nitrate (plot 100)	Ca-sludge (plot 216)	Al-sludge (plot 316)	Fe-sludge (plot 416)
0-1	7.5	19.4	8.6	12.6
1-2	7.1	19.0	8.7	12.0
2-3	6.5	16.4	8.0	12.6
3-5	5.9	15.2	7.0	11.0
5-7	5.4	13.2	6.2	8.9
7-9	5.3	8.2	5.6	6.8
9-12	4.9	6.5	5.2	5.8
12-15	4.6	5.8	4.8	5.4
15-18	4.4	4.9	4.5	5.1
18-22	4.4	4.9	4.7	4.9

Character of the earthworm population

Earthworm biomass and populations in all treatments were dominated by *Lumbricus terrestris* L., which feeds and mates on the soil surface as an adult (but not as a juvenile). It is the only worm species at this site that has the ability to remove soil and litter from the surface and transfer it to subsurface horizons through defecation (casting). Of 173 worms collected from twelve quadrats in 1989, 164 were *L. terrestris* [the other nine were *Aporrectodea turgida* (Eisen) representing 5% of numbers and 1% biomass] that were used for biological and chemical analyses, because of their dominance and anecic habit (Bouché, 1977). De St. Remy and Daynard (1982) collected earthworms from a neighbouring site at the Elora Experimental Station in 1980 and 1981, and observed a similar suite of earthworm species, but *L. terrestris* was less dominant.

In the spring of 1990, *L. terrestris* constituted ~78% of the biomass and 82% of the numbers of collected earthworms. Abundance and biomass of *L. terrestris* populations in 1989 and 1990 for the different treatments is given in Table 2. In both 1989 and 1990, the control plots yielded the lowest numbers and biomass of *L. terrestris*, probably as a consequence of the reduced organic matter inputs compared to sludge-treated plots. Numbers of *L. terrestris* were higher in the spring of 1990 than the previous fall, but biomass figures were higher in the fall of 1989; this effect is due to the higher frequency of juveniles with lower mean weights in spring populations (Table 2).

L. terrestris are abundant on calcareous soils (Wallwork, 1970; Jamieson, 1981), but when we sampled, there were not significantly larger populations of *L. terrestris* on Ca-sludge treatments (Table 2) compared to the other treatments.

There was no statistically detectable toxic effect of heavy metals on *L. terrestris* numbers or biomass that can be distinguished amongst sludge treatments or amongst different levels of the same sludge treatment (the Al-sludge treatments had the highest levels of heavy metal inputs, Table 1).

Distribution of cadmium in soil and earthworms

Cadmium levels in treatment plots 100, 216, 316 and 416 are shown in Table 4. Cadmium input was three times higher in Al-sludge than in Fe-sludge (Table 1). Field residues of Cd in soil were between 1.5 and 23 times higher in the soil of the Al-sludge than in the soil of the Fe-sludge treatment by 1989. Most of the Cd residues were concentrated in the 0–9 cm surface layers of the soil profile in both treatments in association with the higher organic matter content of these horizons. The proportions of Cd applied in the different treatments (3:1 for Al-sludge:Fe-sludge, Table 1) compared closely with the amount of Cd measured and summed from each 1 cm horizon down to 22 cm

TABLE 4

Mass of cadmium in a 22 cm deep soil column with a cross-sectional area of 1 cm² in the three sludge and fertilizer (ammonium nitrate) treatments at Elora, Ontario in 1989

Depth (cm)	Soil Cd residues ($\mu\text{g}/\text{cm}^3$) for indicated treatments			
	Amm. nitrate (plot 100)	Ca-sludge (plot 216)	Al-sludge (plot 316)	Fe-sludge (plot 416)
0-1	0.00	1.80	65.12	5.06
1-2	0.00	2.33	70.50	6.91
2-3	0.21	1.82	74.52	10.00
3-5	1.13	3.88	48.87	20.47
5-7	0.49	2.13	22.68	10.33
7-9	0.51	1.59	8.74	4.66
9-12	0.77	0.00	6.69	4.20
12-15	0.00	0.69	3.80	2.51
15-18	0.00	0.71	3.46	1.84
18-22	0.00	0.00	2.61	1.01
<i>Prorated sum^a</i>				
In $\mu\text{g}/\text{cm}^2$	3.10	14.96	306.98	66.99
In kg/ha	0.31	1.50	30.70	6.70

^aObtained by summing Cd residues for each 1 cm increment through the depth of the 22 cm soil column assuming a 1 cm² surface.

(4.7:1 for Al-sludge:Fe-sludge, Table 4) nearly 10 years after sludge application ended.

Cadmium levels in *L. terrestris* at Elora ranged from 570 mg/kg (ashed wt.) in the Al-sludge plot in 1990, to 27 mg/kg in the Fe-sludge plot (Table 5). The most consistent series is provided from the Al-sludge plots which had the highest inputs of Cd, and where earthworm tissue burdens can be seen to increase with increasing inputs (and soil residues) of Cd. The corollary of this result is that *L. terrestris* individuals migrated little amongst the plots (there was no headland between plots), because 44% of the variance ($r=0.66$) of observed worm tissue burdens of Cd were explained by soil Cd burdens from which the worms were collected. If migration amongst plots was common, we would expect no correlation between soil and earthworm tissue burdens of Cd. This effect can still be clearly discerned 10 years after sludge additions stopped. The levels of soil Cd we observed were similar to those of Levine et al. (1989) following 10 years of sludge application to an old field, but we did not observe concentration of Cd in earthworm tissue above soil concentrations where Cd was present at high levels in soil (Al-sludge treatments). However, where soil-Cd concentrations were observed at lower levels, earthworms concentrated Cd from about five to 200 times above soil concentrations (the other three treatments, Tables 4 and 5).

TABLE 5

Cadmium concentrations ($\mu\text{g Cd/g}$ ashed wt. of earthworm tissue) in *L. terrestris* collected from different sludge and fertilizer treatments at Elora, Ontario on October 14, 1989 (in parentheses) and May 14, 1990

kg N/ha added	Cadmium ($\mu\text{g/g}$)			
	Amm. nitrate	Ca-sludge	Al-sludge	Fe-sludge
0	90 (47) ^a	—	—	—
100	32	—	—	—
200	60	50	140	70
400	90	70	120	200
800	—	70	270	56
1600	—	35 (68) ^a	570 (266) ^a	27 (105) ^a

— = there were no treatment plots for these categories.

^a1989 Cd residue data are means of three determinations.

Comparative analysis of elemental compositions of the earthworm burrow linings and soil matrix (Figs. 1, 2) by PIXE and EMP provided a wealth of data (Tables 6 to 8), which were sorted according their abundance in adjacent matrix soil. Several trends were suggested by the data: excluding Si, elements were nearly always more concentrated in fecal pellets or burrow wall linings (fecal pellets “pasted” on the burrow walls) than in the matrix soil. The data exhibited a general trend to decreasing concentration of trace metals from fecal pellets to inner burrow lining to middle burrow lining to adjacent matrix soil.

Soils and especially earthworm fecal pellets are constituted of discontinuous particulate matter with large areas of voids. Thus the choice of any 5 μm diameter spot for analysis forces an acceptance of high inherent variability (see Table 9 and note high coefficients of variation). Therefore, in this study we used the the raster array capability of PIXE to obtain $400 \times 400 \mu\text{m}$ area analyses of elements. The variation in elemental concentration between spot and area measurements by PIXE can be seen by comparing data in Table 7 to data in Table 9. Although determinations by rastered area analysis of elemental concentrations are less precise than spot measurements, they have demonstrated the consequences of anecic earthworm activity on redistributing elements in burrows and matrix soil (Tables 6 to 8).

Application of large amounts of organic matter to the soil surface from 1973 to 1980 would have induced large increases in densities and individual mean biomasses of *L. terrestris* during the period of application and for some years afterwards (Hamilton and Dindal, 1989). The induction of a large *L. terrestris* earthworm population would have resulted in large increases in worm burrows that would accelerate the movement of leachable metals into the soil aquifer (from increased surface areas of larger numbers of worm burrows,

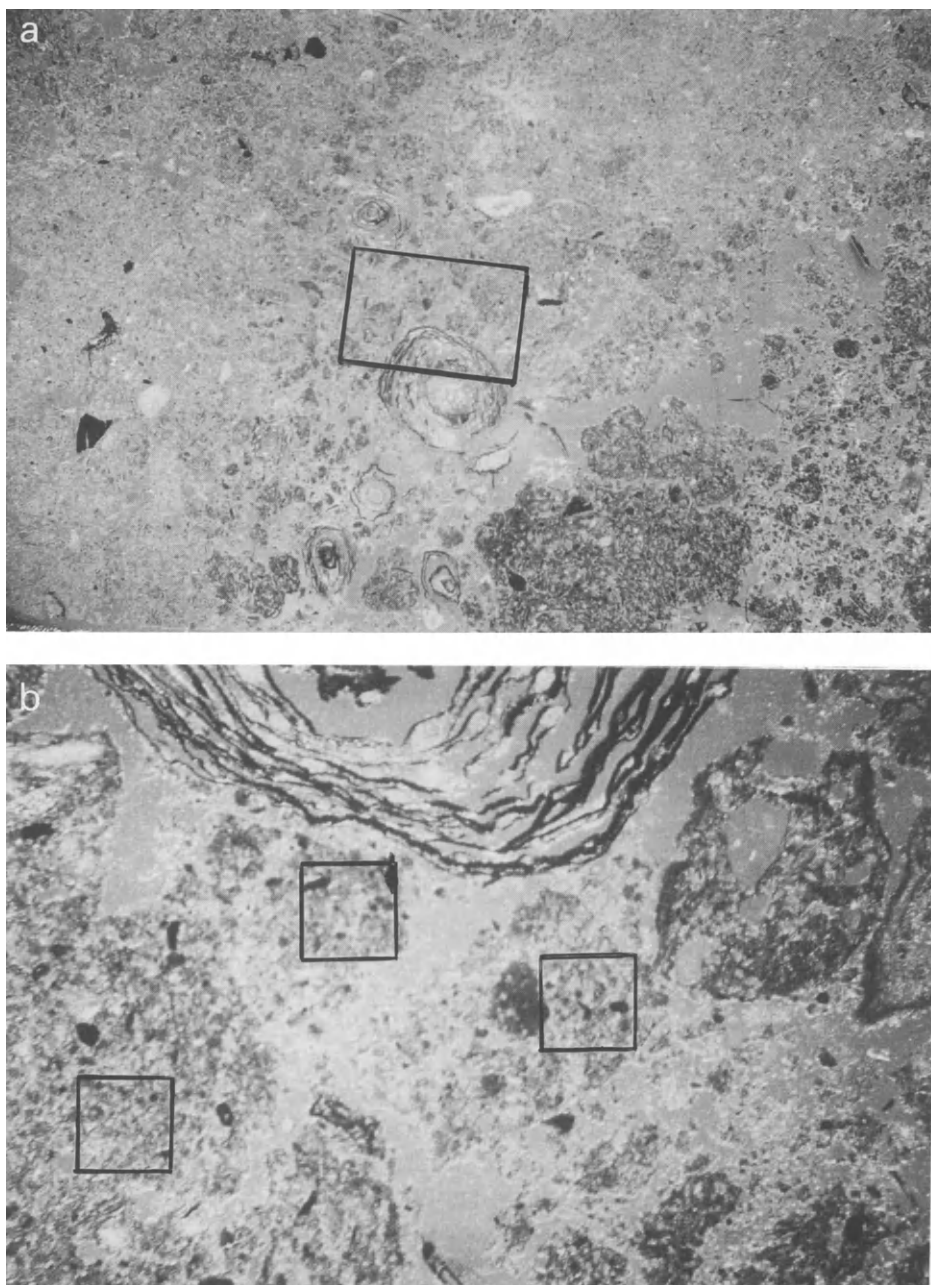


Fig. 1. (a) Photomicrograph of resin-impregnated soil block containing earthworm fecal pellets near soil surface (0–5 cm). Soil was taken from the Fe-sludge plot (416); rectangle is 4 mm long and delineates the total length of (b). (b) Photomicrograph of earthworm fecal material in resin-impregnated soil block; squares are 400 μm on each side, and represent areas exposed to the PIXE beam for elemental analysis.

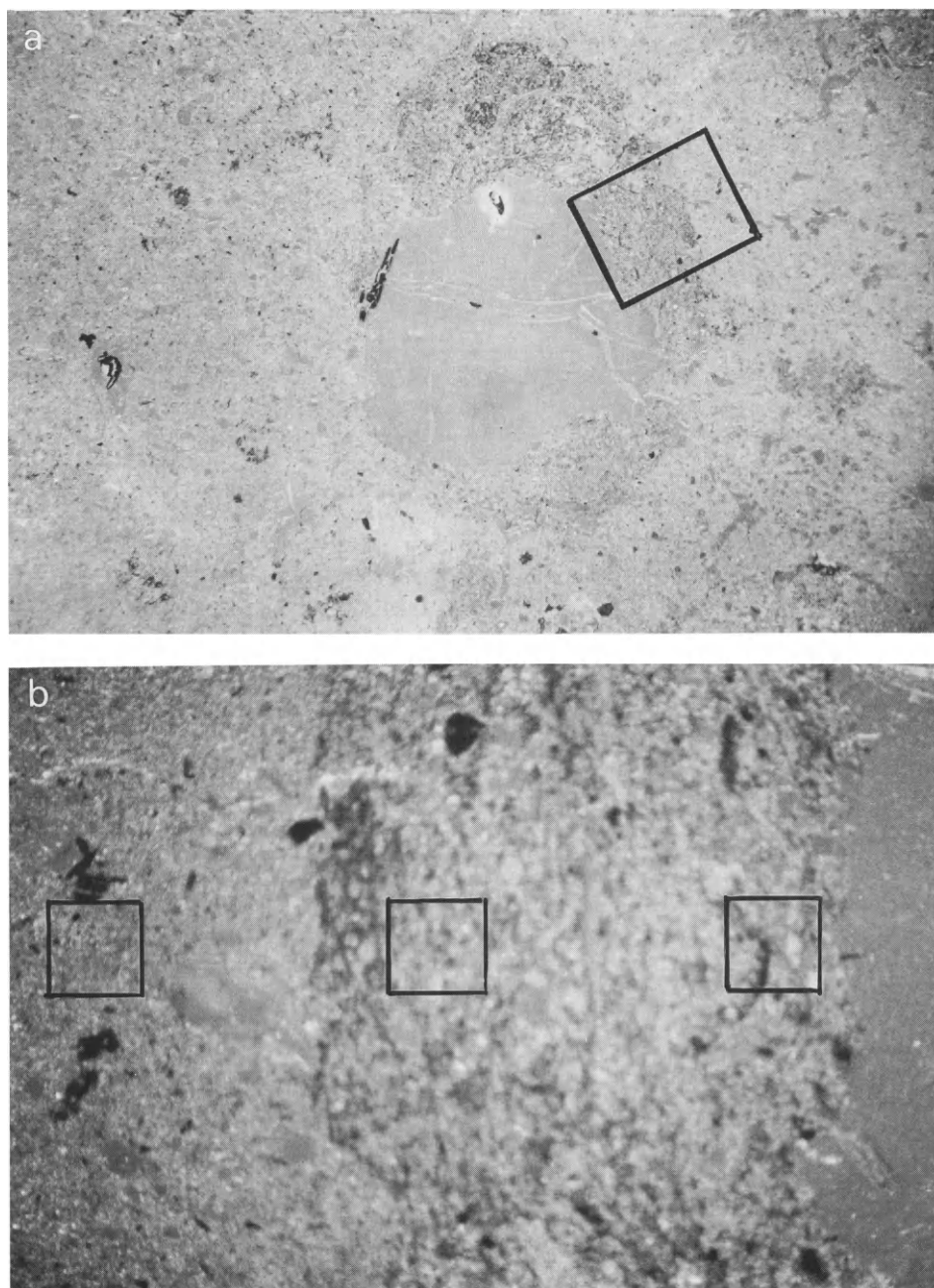


Fig. 2. (a) Photomicrograph of resin-impregnated block taken from Fe-sludge plot (416) containing cross-section of an earthworm burrow at a depth of 40–45 cm in the soil. Rectangle is 4 mm long, and represents the total length of (b). (b) Photomicrograph of resin-impregnated soil block containing earthworm matter lining the burrow wall. The squares are 400 μm on each side, and represent the areas exposed to the PIXE beam for elemental analysis.

TABLE 6

Concentration (mg/kg) of 13 elements as measured by PIXE in $400 \times 400 \mu\text{m}$ areas (raster mode) of selected plasmic features of worm burrows in the Ca-sludge treatment

Elements	Fecal pellets (0–5 cm)	Inner burrow lining (40–45 cm)	Middle burrow lining (40–45 cm)	Adjacent matrix soil (40–45 cm)
Si	147,389	149,497	166,383	98,122
Al	36,676	36,482	46,878	31,404
Fe	30,713	16,566	22,990	10,318
Ca	54,608	30,143	20,819	3124
P	11,364	5687	4501	1192
Mn	972	314	565	309
Zn	822	198	247	77
Co	456	193	247	57
Ni	839	327	353	39
Cr	405	109	161	29
Cu	310	30	48	17
Pb	193	35	45	7
Cd	0	0	0	0

TABLE 7

Concentration (mg/kg) of 13 elements as measured by PIXE in $400 \times 400 \mu\text{m}$ areas (raster mode) of selected plasmic features of worm burrows in the Al-sludge treatment

Elements	Fecal pellets (0–5 cm)	Inner burrow lining (40–45 cm)	Middle burrow lining (40–45 cm)	Adjacent matrix soil (40–45 cm)
Si	89,910	183,804	128,548	177,041
Al	54,429	43,794	31,313	30,959
Fe	91,819	26,398	15,032	14,545
Ca	55,997	29,388	7007	6144
P	73,857	10,310	122	182
Mn	261	730	460	148
Co	757	235	244	108
Ni	263	69	50	37
Zn	5961	544	71	31
Pb	1527	133	16	11
Cu	3793	131	8	7
Cr	5039	613	24	0
Cd	25	0	5	0

and exposure of fecal pellets on the burrow walls to accelerated water flows through the burrows). Larger worm populations would have increased the transfer of sludge from the surface to deeper soil horizons via surface feeding and casting within the soil profile. From the data of Tables 6 to 8, it appears likely that trace elements were leached away from burrow walls more rapidly than from fecal pellets within the surface soil layers.

TABLE 8

Concentration (mg/kg) of 13 elements as measured by PIXE in 400 x 400 μm areas (raster mode) of selected plasmic features of worm burrows in the Fe-sludge treatment

Elements	Fecal pellets (0–5 cm)	Inner burrow lining (40–45 cm)	Middle burrow lining (40–45 cm)	Adjacent matrix soil (40–45 cm)
Si	223,707	176,030	137,626	144,541
Al	57,309	41,849	32,390	33,974
Fe	35,689	22,490	18,556	16,492
Ca	19,153	12,494	9919	7374
P	9531	6255	6418	338
Mn	644	567	449	240
Co	196	262	171	213
Cu	492	103	154	16
Ni	107	77	38	39
Zn	278	74	344	94
Cr	147	59	66	19
Pb	250	57	188	12
Cd	0	0	0	0

TABLE 9

Concentration (mg/kg) of 12 elements, as measured by PIXE in 5 μm spot mode, of selected plasmic features of worm burrows in Al-sludge treatment

Elements	Fecal pellets (0–5 cm)	Inner burrow lining (40–45 cm)	Middle burrow lining (40–45 cm)	Adjacent matrix soil (40–45 cm)
Al	83,088 (44)	44,299 (57)	53,191 (44)	8404 (34)
Fe	104,865 (46)	47,397 (31)	41,876 (46)	5049 (18)
Ca	137,354 (68)	47,258 (89)	61,510 (64)	1252 (80)
P	150,184 (68)	54,525 (117)	53,767 (73)	227 (113)
Cr	10,501 (70)	2703 (110)	24 (141)	41 (7)
Co	884 (39)	506 (43)	504 (24)	34 (141)
Zn	2834 (78)	1174 (96)	286 (28)	19 (42)
Ni	458 (64)	171 (125)	164 (33)	17 (141)
Cu	2028 (38)	512 (86)	41 (27)	3 (33)
Pb	765 (57)	307 (124)	43 (44)	3 (73)
Cd	16 (120)	6 (78)	4 (141)	3 (141)
Mo	94 (120)	17 (125)	1 (141)	1 (72)

Coefficients of variation in parentheses; $n=9$ for fecal pellets; $n=3$ for burrow linings and adjacent soil.

CONCLUSIONS

Even though sludge and fertilizer treatments had been suspended by 1980, statistically significant effects on earthworm biomass attributable to sludge treatments were still observable in 1989 and 1990.

Earthworm populations were stable even within very small areas because soil Cd levels still explained 44% ($r=0.66$) of worm Cd residues taken from specific treatment areas 10 years after Cd application ended (i.e., *L. terrestris* earthworms were not subject to extensive dispersal).

Table 9 shows the coefficient of variation for detecting the various elements ranging from 7 to 141% using PIXE in the 5 μm spot mode. The high coefficient of variation was due to the high variability of elemental distributions from particle to particle within the soil matrix; the microfabric is very heterogeneous at this scale, and mapping distributions of elements in soil horizons at microscopic scale will yield more variable data than traditional wet chemistry techniques on composite samples.

Following the movement and distribution of elements in soil at the scale of microfabric will be of great value in detecting and unravelling biotic and abiotic processes and interactions in soil. The power of the microscopic and submicroscopic techniques will be further enhanced by combining elemental analyses with visual identification techniques (Lussenhop et al., 1991) of biotic and abiotic pedo-features to establish the functions and contributions of biota to soil processes. Soil microfabric is a complex ecosystem, but the tools to observe and measure biotic processes are at hand and rapidly improving.

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Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils

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ABSTRACT

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The effect of soil type on carbon (C) and nitrogen (N) mineralization rates in grassland soils was investigated along with the physical and biological soil characteristics that may have caused the observed differences in mineralization rates between soil types.

The percentage of mineralized organic N was higher in sandy soils than in loams and clays; this was not observed for C. In loams and clays small pores constituted a higher percentage of the total pore space than in sandy soils. Two mechanisms of physical protection of organic N were distinguished. In clay soils physical protection of organic material by its location in small pores was the main mechanism. In sandy soils, however, organic material was protected by its association with clay particles. In loams both mechanisms played a role. The protected organic material associated with clay particles consisted of amorphous undefined material that did not stain with acridine orange, indicating a high degree of decomposition, while the non-protected organic material present in the sand fraction consisted of plant debris that stained intensely with acridine orange. Physically protected organic matter had a lower C/N ratio than organic matter that was not physically protected.

Grazing pressure on bacteria by bacterivorous nematodes was higher in sandy soils than in loams and clays. This coincided with a higher N mineralization rate per bacterium. The C/N ratio of the microbial biomass was higher in sandy soils than in loams and clays and was positively correlated with the N mineralization rate per unit of microbial biomass N. This is in agreement with the concepts of food webs that N mineralization is positively correlated with the C/N ratio of the consumer (bacteria) for a given C/N ratio of the substrate (organic matter). It is not yet clear which of the factors investigated are the most important in determining N mineralization rates in grassland soils.

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INTRODUCTION

Net mineralization of soil organic matter and decomposition of plant material is more rapid in sandy soils than in clay soils (Catroux et al., 1987; Hassink et al., 1990; Ladd et al., 1990; Verberne et al., 1990). The lower net mineralization in clay soils may be partly caused by a greater physical protection of soil organic matter and microbial biomass (Verberne et al., 1990). Different mechanisms have been suggested to explain the phenomenon of physical protection in soils: (i) adsorption of organics to surfaces of clays or coating of organics by clay particles (Tisdall and Oades, 1982; Fig. 1), and (ii) entrapment of organics in small pores in aggregates inaccessible to microbes (Elliott and Coleman, 1988; Fig. 2).

Soil structure may also control decomposition and mineralization processes by its effect on grazing intensity on microbes. Predation of microbes by protozoa and nematodes has been suggested as an important mechanism of nutrient turnover in soil (Coleman et al., 1978; Elliott et al., 1980). A large proportion of bacteria may occupy pores $< 3 \mu\text{m}$ (Kilbertus, 1980), while protozoa and nematodes are restricted to larger pores. This means that a large part of the bacterial population will be physically separated from the protozoa and nematodes in soil (Postma and Van Veen, 1990).

As the relative amount of large pores is usually higher in sandy soils than in loamy and clay soils (Papendick and Campbell, 1981), predation is expected to be more intense in sandy soils than in loamy and clay soils (Heijnen et al., 1988). The results of Hassink et al. (1993) indicated that the higher

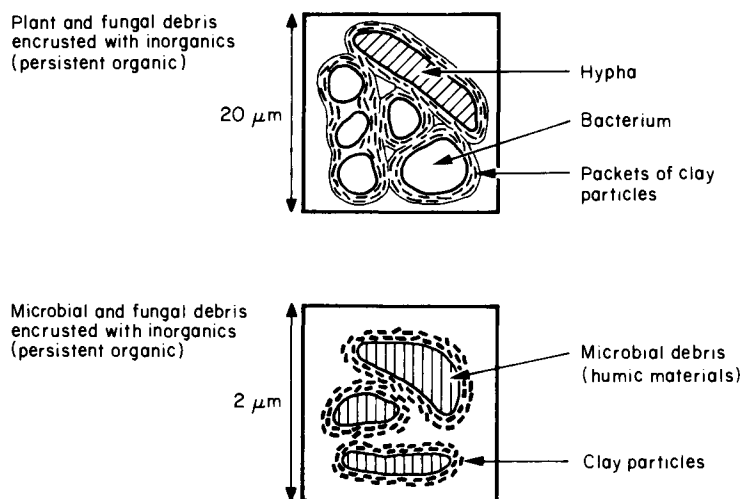


Fig. 1. Part of the model of aggregate organization as given by Tisdall and Oades (1982).

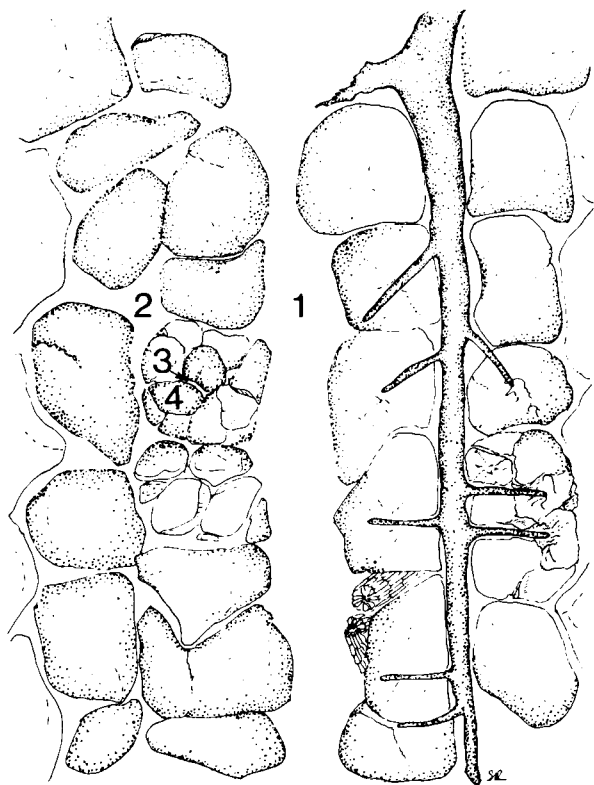


Fig. 2. Hierarchical class of soil pore space according to Elliott and Coleman (1988). 1 = macropores, 2 = pores between macroaggregates, 3 = pores between microaggregates, 4 = pores within microaggregates.

grazing pressure in sandy soils might strongly affect the N mineralization rate.

Soil structure might also affect the relative contribution of bacteria and fungi to the total microbial biomass. Fungi can be present in pores with sizes different from those where bacteria are found, as hyphae can vary considerable in diameter and are not restricted to water films. It is generally assumed that the C/N ratio of fungi is higher than that of bacteria (Hunt et al., 1987). The amount of N mineralized by microbes will generally be higher in soils which contain a microbial population with a high C/N ratio than in soils with a microbial population with a low C/N ratio. This means that the contribution of fungi to the total microbial biomass may affect N mineralization rates.

The aim of the present study is to describe to what extent sandy soils, loams and clays differ in the above-mentioned soil physical and biological characteristics and how this affects C and N mineralization rates.

METHODS

Field description

In March 1989 and May 1991 samples were collected from grassland soils which had been under grass for at least 30 years. In Swifterbant the old sward was reseeded in 1985. The grasslands were grazed by dairy cattle and received 400–500 kg fertilizer-N per ha per year. Samples were taken from the 0–10 cm layer. Some characteristics of the soils are given in Table 1.

Measurements

The following measurements were performed in May 1991:

- (1) potential C and N mineralization rates,
- (2) pore size distribution,
- (3) increase in C and N mineralization rates due to disruption of soil structure,
- (4) amount and microscopic characteristics of C and N associated with particles $< 2 \mu\text{m}$ (clay) and of C and N present in the sand fraction $> 200 \mu\text{m}$ (macro organic matter),
- (5) biomass of bacteria, fungi, protozoa and nematodes (direct counts), and
- (6) microbial biomass C and N according to the fumigation incubation method.

In March 1989 only the potential mineralization rates and microbial biomass according to the fumigation method were determined.

TABLE 1

Some soil characteristics of the 0–10 cm layer of the grassland sites studied

	C N		C/N (g kg ⁻¹)	pH KCl	Granular composition, % particles			Bulk density (g cm ⁻³)
	(g kg ⁻¹)				< 2 μm	< 16 μm	< 50 μm	
<i>Sand</i>								
Heino	19.8	1.1	18.0	4.8	1.9	2.8	8.7	1.33
Tynaarlo	43.8	2.5	17.5	4.4	2.4	4.4	23.5	1.25
<i>Loam</i>								
Swifterbant	17.7	1.5	11.8	7.0	23.0	38.4	72.2	1.22
Burum	53.7	5.5	9.8	4.9	24.1	36.5	71.7	1.09
<i>Clay</i>								
Zaltbommel	60.7	6.6	9.2	5.4	51.1	76.0	86.4	1.05
Haskerdijk	56.0	5.1	11.0	4.9	54.0	77.2	88.5	1.00

Description of the methods

Three mixed samples, each consisting of 20 bulked cores, were taken from every location at each sampling date for the measurements of microbial biomass, bacterial, fungal and protozoal biomass and C and N mineralization. The samples were sieved through a 0.008 m mesh screen; roots and stubble were removed and the samples were analyzed separately. In all soils the moisture content was close to 60% of the water holding capacity at both sampling times. Field-moist samples were incubated.

Potential C and N mineralization rates

N mineralization was determined by measuring the increase in mineral N after incubation of soil samples in glass jars at 20°C for 12 weeks. Drying of the samples was prevented by covering the jars with a seal permeable to air, but impermeable to water. Mineral N was measured colorimetrically after extraction with 1N KCl solution for 1 h using a soil: water ratio of 1:2.5.

C mineralization was measured by incubating soil samples in 1.5 l airtight jars containing a vial of 10 ml 0.5M NaOH. At the sampling dates, the trapped CO₂ was measured after precipitating the carbonate with excess BaCl₂. The CO₂ produced in the period between 10 and 20 days after the start of the incubation was used to calculate the C mineralization rate. A flush in CO₂ production was observed during the first 10 days after incubation, probably caused by the disturbance of the soil.

Pore size distribution

The relationship between soil water potential and moisture content was determined according to Klute (1986) in undisturbed soil samples from the 2.5–7.5 cm layer. The effective pore neck diameter (d) was estimated from the water retention curve as:

$$d = 2r = -30.0 \times 10^{-6} h^{-1} \text{ (m)} \quad (1)$$

where h is the pressure head, r is the radius of the curvature of the capillary pore (m). Equation (1) has been derived for a water temperature of 15°C (Vargas and Hattori, 1986). The corresponding pF values can be obtained by taking the logarithm of the absolute value of the pressure head: $\text{pF} = {}^{10}\log [h]$. The pore volumes corresponding with different pore neck diameters can then be calculated from the retention curve.

Increase in C and N mineralization after disruption of soil structure

The C and N mineralization rates were measured in soil samples that had been pressed through a 0.001 m mesh size screen (fine sieving) and in soil samples that had been sieved through a 0.008 m mesh size screen (coarse sieving). It was assumed that the increase in mineralization observed after fine sieving is caused by the release of a part of the physically protected organic matter, located in small pores. The relative increase in mineralization due to fine sieving in comparison with the mineralization rate in coarsely sieved samples during the same period then gives a qualitative index of the extent of physical protection of organic matter. For C the increase was determined during the first five days after the start of the incubation; for N a period of two weeks was taken (the lengths of the periods the increases in mineralization after fine sieving remained for C and N; Hassink, 1992).

Amount and characteristics of C and N associated with soil particles < 2 μm and present in macro-organic matter

Dry soil (50 g) was suspended in 250 ml water for 24 hours. The samples were treated ultrasonically for 15 minutes with a probe-type ultrasound generating unit (Soniprep 150). Probe output was calibrated by measuring the temperature produced upon ultrasonifying a known mass of water for a specified time (North, 1976). Output power assessed was 30 W. Heat buildup in the soil suspension was controlled by a water cooling jacket. The dispersed soil suspension was transferred to a 1 l glass cylinder. The cylinder was shaken end over end until the soil was suspended. A table showing the settling-times for 2 μm particles at temperatures between 15 and 25°C was constructed by applying Stoke's law and a particle density of 2.675 g cm⁻¹. After the correct settling time, particles < 2 μm were isolated by siphoning the suspension at the appropriate depth. The fractions were dried for 24 hours at 105°C.

Macro-organic matter is defined as organic material not closely associated with the soil mineral fraction. The macro-organic matter was separated from 100 g portions of soil that had been sieved through a 0.008 m size screen and was cleared from visible root and plant parts. The samples were dispersed in pyrophosphate solution for 2 hours. The suspensions were shaken for 1 h and sieved through a 200 μm mesh screen. The sand and the macro-organic matter fraction was dried overnight, ground and analysed for total C and N using a Carlo Erba NA 1500 analyzer.

Microscopic characterization of organic matter associated with soil particles < 2 μm and present in the macro-organic matter

Subsamples of both the < 2 μm soil particles and the macro-organic matter fractions were examined using a Confocal Laser Scanning Microscope (CLSM,

Leica, Heidelberg, Germany). Of each fraction a few milligrams were suspended in 2 ml demineralized water, fixed with 4% (wt/vol., final concentration) formaldehyde, and stained for 5 min with 0.05% (wt/vol., final concentration) acridine orange (Faegri et al., 1977), for 20 min with 0.0001% 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) in 0.05M Na_2HPO_4 buffer at pH 9. The particles were collected on a 0.2 μm pore size, 25 mm diameter aluminium oxide membrane filter (Anodisc 25, Anotec Separations Ltd, Banbury, Oxon, England), and rinsed with 5 ml distilled water. The filter was mounted with immersion oil between a glass slide and a cover glass. The cover glass was sealed with nail polish. Under the CLSM the specimen was excited at 488 and 514 nm by an argon ion laser (2–50 mW). On the first channel the fluorescence of acridine orange or DTAF was detected using a beam splitter of 510 nm and a 525 nm bandpass barrier filter. The reflected light was measured simultaneously on the second channel. With reflected light both fluorescent and non fluorescent particles were detected. The acridine orange or DTAF fluorescence was used to obtain images of organic residues. The obtained video images were photographed using a Polaroid FreezeFrame Video Recorder (Polaroid Co., Cambridge, MA, USA).

Biomass of bacteria, fungi, protozoa and nematodes

Bacteria. Bacteria were counted in europium-chelate-stained soil smears using a Leitz epifluorescence microscope (magnification $\times 1000$) equipped with a HBO-50 mercury lamp. A 1:10 diluted soil suspension was homogenized in a blender for 30 seconds. Details about the method are given by Hassink et al. (1991). The average volume of a bacterium was assumed to be 0.2 μm^3 (Bloem, unpubl. results). It was assumed that the bacteria contained $320 \times 10^{-15} \text{ g C } \mu\text{m}^{-3}$ (Van Veen and Paul, 1979).

Fungi. Fungal biomass was estimated by measurements of hyphal length in agar films, made by mixing 1 ml of a 1:10 diluted soil suspension and 6 ml of agar, stained with fluorescent brightener (Hassink et al., 1991). A conversion factor of 0.33 (ratio between dry mass and wet volume) was used for biomass calculations and a carbon content of 40% of the dry weight (Van Veen and Paul, 1979).

Protozoa. Protozoa were enumerated by the most probable number method after Darbyshire et al. (1974). Details are given by Brussaard et al. (1990).

Nematodes. Three 200 g samples were taken from every location with a spade. Roots were separated by hand and nematodes were isolated from field-moist soil by elutriation (Oostenbrink, 1960). For the isolation of nematodes from the roots, roots were spread over a nematode filter and nematodes were

allowed to creep through the filter during 48 hours at 18 °C. The nematode suspensions from soil and roots were counted separately under a stereo microscope and 60 specimens per sample were identified under a high power microscope. Four different feeding categories were distinguished: bacterivorous, omnivorous, fungivorous and phytophagous nematodes. Details about conversion factors are given by Brussaard et al. (1990).

Microbial biomass C and N. The amounts of C and N in the microbial biomass were determined by the chloroform fumigation incubation technique (Jenkinson and Powlson, 1976). The exact procedure has been described by Hassink et al. (1991). For C a k value of 0.45 and for N a k value of 0.4 was used to calculate the biomass C and N from the flush (Jenkinson and Ladd, 1981; Schnürer et al., 1985).

Statistical treatment

Differences in mineralization rates between soils and treatments (fine sieving in comparison with coarse sieving) were analysed with the t -test. The relations of pore size distribution with increase in mineralization after fine sieving; biomass of bacteria, fungi, protozoa and nematodes with pore size distribution; mineralization per unit of bacterial or microbial biomass with grazing intensity and C:N ratio of the microbial biomass were analysed with regression analysis (Genstat manual, 1987).

RESULTS

C and N mineralization rates

C mineralization rates were generally higher in loams and clays than in sandy soils; however, the percentage of mineralized organic C was not significantly different for the different soil types (Table 2). The percentage of organic C mineralized per day fluctuated considerably between sampling dates and locations. The percentage of mineralized organic C was highest in Swifterbant, the value for this location may, however, be an overestimation because it is a calcareous soil and part of the CO₂ will originate from carbonate.

The N mineralization rates ranged between 0.7 and 3.1 kg ha⁻¹ day⁻¹ (Table 2). The percentage of mineralized soil organic N per day was significantly ($p < 0.05$) higher in sandy soils than in loams and clays at both sampling dates.

Pore size distribution

The total pore space was lowest in the sandy soils and highest in the clayey soils. In the loamy and clay soils, the pores with diameters $< 0.2 \mu\text{m}$ were

TABLE 2

Mineralization rates of C (period 10–20 days after the start of incubation) and N (period 0–84 days after the start of the incubation) at 20°C ($\text{kg ha}^{-1} \text{ day}^{-1}$ and % of total soil C and N mineralized per day $\times 100$)

	Carbon				Nitrogen			
	$\text{kg ha}^{-1} \text{ day}^{-1}$		% ($\times 10^2$)		$\text{kg ha}^{-1} \text{ day}^{-1}$		% ($\times 10^2$)	
	1989	1991	1989	1991	1989	1991	1989	1991
<i>Sand</i>								
Heino	20.6	11.6	7.8	4.4	1.1	1.2	7.3	8.3
Tynaarlo	26.2	19.6	4.9	3.6	1.4	1.9	4.5	6.0
<i>Loam</i>								
Swifterbant	23.1	24.6	10.5	11.2	0.7	0.7	3.8	3.6
Burum	44.3	51.0	7.2	8.3	2.3	2.6	3.6	4.1
<i>Clay</i>								
Zaltbommel	60.0	28.8	8.2	4.0	2.1	1.8	2.7	2.2
Haskerdijk	–	41.6	–	6.2	–	3.1	–	5.0

TABLE 3

Pore size distribution of soils

	Pore size distributions (% of soil volume)							Total
	<0.2	0.2–1.2	1.2–6	6–30	30–90	90–150	>150	
<i>Sand</i>								
Heino	6.0	2.1	8.3	18.8	5.4	1.3	3.6	42.2
Tynaarlo	10.5	5.1	10.1	17.0	3.7	0.2	2.7	49.3
<i>Loam</i>								
Swifterbant	18.4	8.3	13.4	0.0	3.0	1.5	3.6	48.1
Burum	20.1	11.4	16.1	0.0	2.6	2.0	6.3	58.7
<i>Clay</i>								
Zaltbommel	29.2	10.9	12.9	1.9	0.0	0.0	7.2	62.0
Haskerdijk	30.7	10.3	13.3	0.0	0.7	1.2	5.6	61.9

predominant (Table 3). The pores with diameters between 0.2 and 1.2 μm were more abundant in the loamy and clay soils than in the sandy soils. In the sandy soils, however, the pores with diameters between 6 and 30 μm were most abundant; the pore size class between 30 and 90 μm also made up a larger part of soil volume in the sandy soils than in the loamy and clay soils (Table 3).

TABLE 4
Relative increase in N and C mineralization rates after fine sieving in comparison with coarse sieving (%)

	Increase in mineralization rate	
	C	N
<i>Sand</i>		
Heino	-25	-18
Tynaarlo	-20	-26
<i>Loam</i>		
Swifterbant	16	66*
Burum	65*	21*
<i>Clay</i>		
Zaltbommel	131*	249*
Haskerdijk	13	240*

*Statistical significant different from 0 ($p < 0.05$).

rel. increase in N mineralization (%)
with fine sieving

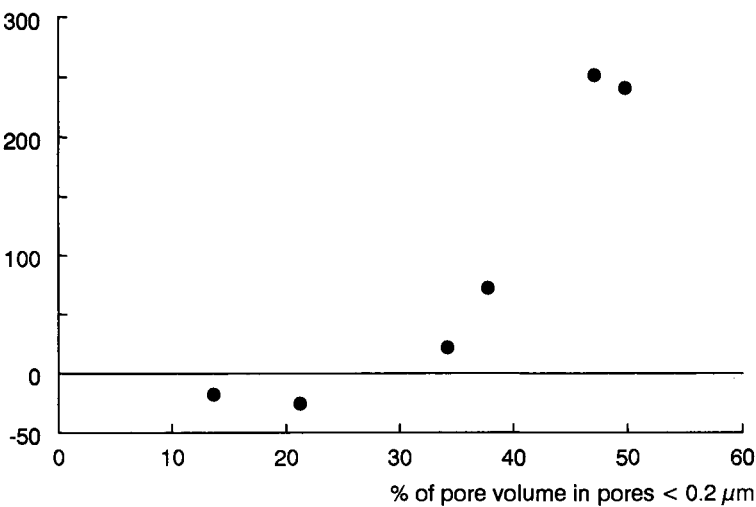


Fig. 3. Relation between the relative increase in N mineralization after fine sieving and the % of soil pore space enclosed by pores with diameters < 0.2 μm. Relative increase is $25(6.8) + 0.10(0.02) \times \% \text{ pore space} < 0.2 \mu\text{m}$; $r^2 = 0.81$ (Hassink, 1992).

Relative increase in mineralization rate after fine sieving; estimation of the amount of physically protected organic matter in small pores

In the loams and clays a relative increase in C and N mineralization due to fine sieving in comparison with coarse sieving was observed (Table 4). In the sandy soils, however, the mineralization rates of both C and N were decreased by approximately 20% (Table 4) after fine sieving; the decrease was not significant ($p < 0.05$). The increase in N mineralization due to fine sieving was largest in the clays. Here the relative increase in N mineralization after fine sieving was larger than the relative increase in C mineralization. This was not the case in the loams. The increases in N mineralization was significant in both loams and both clays ($p < 0.05$); the increase in C mineralization was only significant in one of the loams and one of the clays ($p < 0.05$). There was a good correlation between the relative increase in N mineralization after fine sieving and the percentage of soil pore space occupied by pores with diameters $< 0.2 \mu\text{m}$ ($R^2 = 0.81$; Fig. 3). The correlation with any other pore size class was poor. For C this relationship with pores $< 0.2 \mu\text{m}$ was less clear.

C and N associated with particles $< 2 \mu\text{m}$ and present in the macro organic matter

The C and N content of the clay fraction ($< 2 \mu\text{m}$) was much higher in the two sandy soils than in the loams and the clay (Table 5). Although the total C and N content of the sandy soils differed considerably (Table 1), the C and N content of their clay fraction was the same. In the loams this was different;

TABLE 5

C and N contents and the C/N ratios of the fraction $< 2 \mu\text{m}$, and the sand fraction $> 200 \mu\text{m}$ (containing macro organic matter); and the ratio N content of the fraction $< 2 \mu\text{m}$:N content of the total soil and N content of the fraction $> 200 \mu\text{m}$:N content of the total soil.

	Fraction $< 2 \mu\text{m}$		Ratio		Fraction $> 200 \mu\text{m}$		Ratio	
	C (g kg ⁻¹)	N (g kg ⁻¹)	C/N	N	C (g kg ⁻¹)	N (g kg ⁻¹)	C/N	N
<i>Sand</i>								
Heino	145.4	16.2	9.0	14.7 ^a	15.4	0.9	18.4	0.8 ^c
Tynaarlo	149.6	14.6	10.3	5.8 ^a	20.8	1.2	16.9	0.5 ^c
<i>Loam</i>								
Swifterbant	27.4	2.7	10.2	1.8 ^b	58.8	3.6	16.2	2.4 ^b
Burum	70.3	7.7	9.1	1.4 ^b	123.4	6.4	19.4	1.2 ^b
<i>Clay</i>								
Zaltbommel	37.0	4.4	8.4	0.7 ^b	117.8	7.3	16.3	1.1 ^b
Haskerdijk	—	—	—	—	104.7	8.2	12.7	1.6 ^b

Values with a different character differ significantly ($p < 0.05$).

the loamy soil from Burum which had a higher organic C and N content than the loamy soil from Swifterbant (Table 1), also had a higher C and N content in the clay fraction (Table 5). In all soils the organic material associated with the clay fraction had a C/N ratio between 8.4 and 10.3. In the sandy soils, the C/N ratio of this fraction was considerably lower (significant at $p < 0.05$) than the C/N ratio of the total organic matter. In the loams and the clay soil, however, the C/N ratio of the organic material associated with the clay fraction had almost the same C/N ratio as the C/N ratio of the total organic matter. In the sandy soils the N content of the clay fraction was 5.8–14.7 times higher than the N content of the total soil (Table 5). In the loams this was only 1.4–1.8 times higher and in the clay soil the N content of the clay fraction was even lower than the N content of the total soil.

In the loams and clays, the N content of the sand fraction $> 200 \mu\text{m}$, containing the macro-organic matter, was higher than the N content of the total soil (Table 5). In the sandy soils, however, it was the opposite (Compare Tables 1 and 5). In all soils the C/N ratio of the macro organic matter present in the fraction $> 200 \mu\text{m}$, was between 16 and 19, except for the soil from Haskerdijk (Table 5).

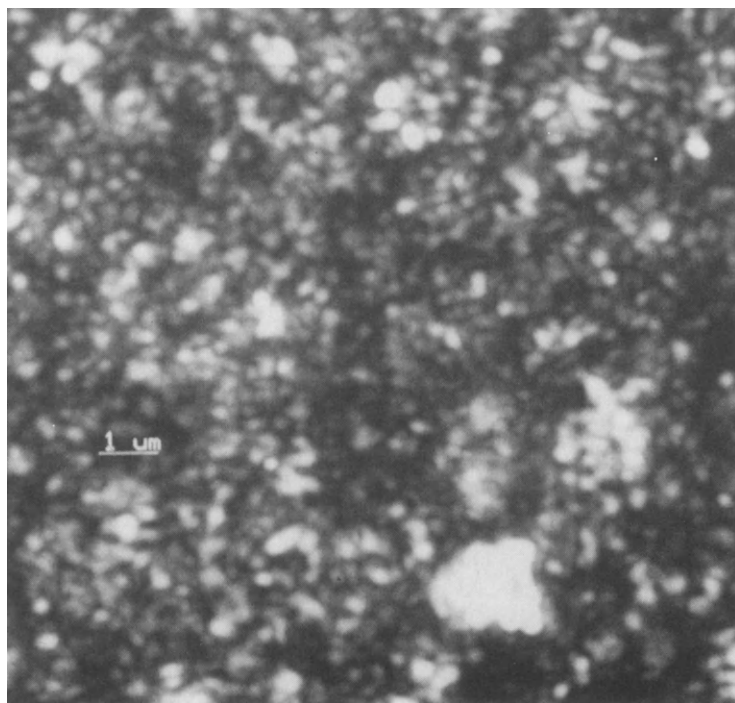


Fig. 4. Image obtained with reflected light of the clay fraction with associated organic matter isolated from the clay soil of Zaltbommel, consisting of undefined particles with a diameter of about $0.3 \mu\text{m}$.

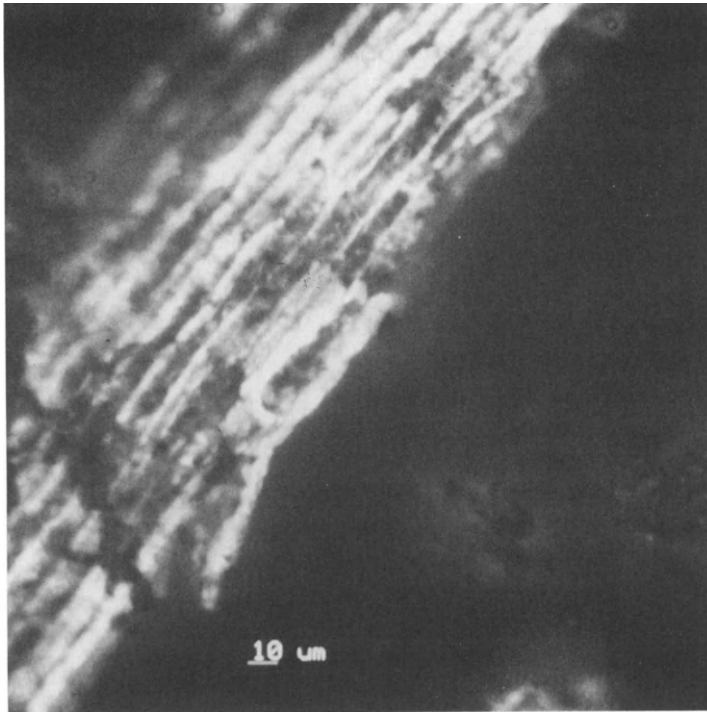


Fig. 5. Image obtained after staining with acridine orange and DTAF of the macro organic matter present in the sand fraction $> 200 \mu\text{m}$ isolated from the clay soil of Zaltbommel. The macro organic matter consists of recognizable plant residues.

Microscopic characterization of organic matter associated with soil particles $< 2 \mu\text{m}$ and present in the macro-organic matter

The organic material associated with the clay fraction did not show any staining by acridine orange or DTAF. Images obtained with reflected light showed that this fraction consisted of particles with a diameter of about $0.3 \mu\text{m}$ (Fig. 4).

The macro-organic matter present in the sand fraction consisted of recognizable plant residues (Fig. 5). This plant debris stained intensely with acridine orange and DTAF.

Biomass C present in various functional groups and its relation with pore size distribution

Bacteria constituted by far the largest biomass pool. In the sandy soils the amount of bacterial biomass was between 126 and 208 kg C ha^{-1} . In the loams and clays, the amount of bacterial biomass was higher: between 250 and 432

TABLE 6

Amount of biomass (kg C ha^{-1}) of functional groups at different sites in the top 10 cm in May 1991

	Bacteria	Fungi	Protozoa		Nematodes			
			a	fl	b	f	p	o
<i>Sand</i>								
Heino	126.0	3.69	2.95	0.32	0.70	0.08	0.19	0.16
Tynaarlo	207.8	3.80	2.90	0.43	0.40	0.04	0.26	0.03
<i>Loam</i>								
Swifterbant	250.0	5.47	5.38	0.90	0.08	0.26	0.20	0.08
Burum	432.8	5.00	4.83	0.87	0.28	0.07	0.23	0.03
<i>Clay</i>								
Zaltbommel	425.8	5.33	5.27	0.96	0.26	0.07	0.05	0.01
Haskerdijk	407.8	4.95	10.81	1.47	0.43	0.07	0.00	0.00

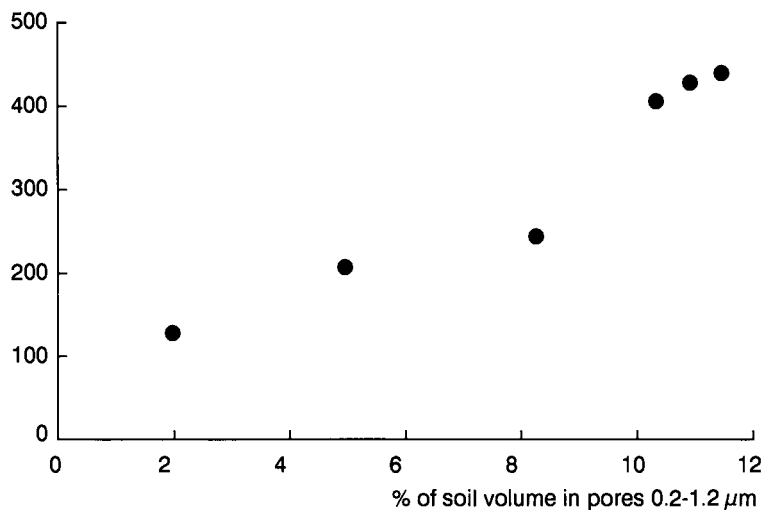
a = amoebae, fl = flagellates, b = bacterivorous, f = fungivorous, p = phytophagous, o = omnivorous.

kg C ha^{-1} (Table 6). The amount of C in the fungal biomass was very low: less than 3% of the amount present in the total microbial biomass. There was no difference in the contribution of fungal biomass to total microbial biomass between the sandy soils, the loams and the clays. The biomass of protozoa was also small, it was generally higher in the loams and clays than in the sandy soils. The biomass of nematodes was even lower than the biomass of protozoa. The biomass of bacterivorous nematodes was relatively high in the sandy soils; the ratio biomass of bacterivorous nematodes:biomass of bacteria was higher in the sandy soils than in the loams and clays. There was a good correlation between the amount of bacterial biomass and the soil volume made up by pores with diameters between 0.2 and $1.2 \mu\text{m}$ ($R^2 = 0.91$) and between the biomass of nematodes and the soil volume made up by pores with diameters between 30–90 μm ($R^2 = 0.84$; Fig. 6). The biomass of fungi and protozoa showed a poor correlation with any of the pore size classes.

Composition and quality of the microbial biomass determined by the fumigation incubation method

The amount of C present in the microbial biomass ranged from 419 to 1869 kg C ha^{-1} (Table 7). These values are much higher than those obtained by microscopic counting. It fluctuated considerably between the two sampling dates. The amount of N present in the microbial biomass ranged from 52 to 553 kg N ha^{-1} (Table 7). The amount of biomass N was lowest in the sandy soils and highest in the clays. The fluctuation between the two sampling dates was much smaller than for C. At both sampling dates the C/N ratio of the

biomass bacteria (kg C/ha)



biomass nematodes (kg C/ha)

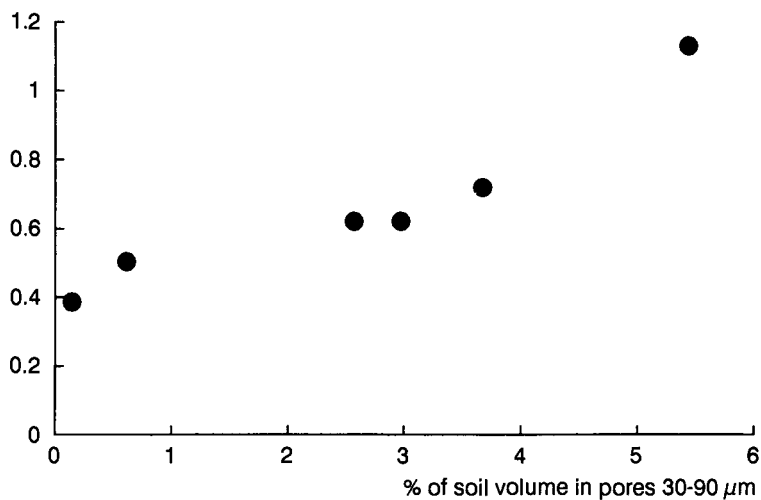


Fig. 6. Relation between the biomass of bacteria (kg C ha^{-1}) and the % of soil volume enclosed by pores with diameters between 0.2–1.2 μm (top: bacterial biomass is $35.4 (40.4) + 34.2 (4.6) \times \%$ of soil volume in pore class 0.2–1.2 μm ; $r^2=0.91$; Hassink et al., 1993); and the relation between the biomass of nematodes (kg C ha^{-1}) and the % of soil volume enclosed by pores with diameters between 30–90 μm (bottom: biomass of nematodes is $0.35 (0.07) + 0.12 (0.02) \times \%$ of soil volume in pore class 30–90 μm ; $r^2=0.84$; Hassink et al., 1993).

TABLE 7

Amount of microbial biomass C and N (kg ha^{-1}) and the C/N ratio of the microbial biomass determined with the fumigation incubation method

	Biomass C		Biomass N		C/N ratio	
	1989	1991	1989	1991	1989	1991
<i>Sand</i>						
Heino	714	419	52	68	13.8	6.2
Tynaarlo	577	1143	65	113	8.9	10.1
<i>Loam</i>						
Swifterbant	803	507	205	218	3.9	2.3
Burum	811	1084	265	215	3.1	5.0
<i>Clay</i>						
Zaltbommel	1027	1869	375	327	2.7	5.7
Haskerdijk	—	1776	—	553	—	3.2

TABLE 8

Mineralization rates of C and N ($\text{kg ha}^{-1} \text{ day}^{-1} \times 10^2$) divided by the amount of bacterial biomass (microscopic counting in kg C ha^{-1}); and mineralization rates of C and N ($\text{kg ha}^{-1} \text{ day}^{-1} \times 10^2$) divided by the amount of microbial biomass C or microbial biomass N (fumigation incubation in kg C or N ha^{-1})

	C and N mineralization rates					
	:Bacterial biomass C		:Microbial biomass C		:Microbial biomass N	
	1991	1991	1989	1991	1989	1991
	C	N	C	C	N	N
	$(\text{kg ha}^{-1} \text{ day}^{-1} \times 10^2)$		$(\text{kg ha}^{-1} \text{ day}^{-1} \times 10^2)$		$(\text{kg ha}^{-1} \text{ day}^{-1} \times 10^2)$	
<i>Sand</i>						
Heino	9.2	0.96	2.9	2.8	2.0	1.8
Tynaarlo	9.4	0.89	4.5	1.7	2.1	1.6
<i>Loam</i>						
Swifterbant	9.8	0.27	2.9	4.9	0.3	0.3
Burum	11.8	0.59	5.5	4.7	0.9	1.2
<i>Clay</i>						
Zaltbommel	6.8	0.42	5.8	1.5	0.6	0.5
Haskerdijk	10.2	0.75	—	2.3	—	0.6

N mineralization/biomass bacteria ($\times 10^2$)

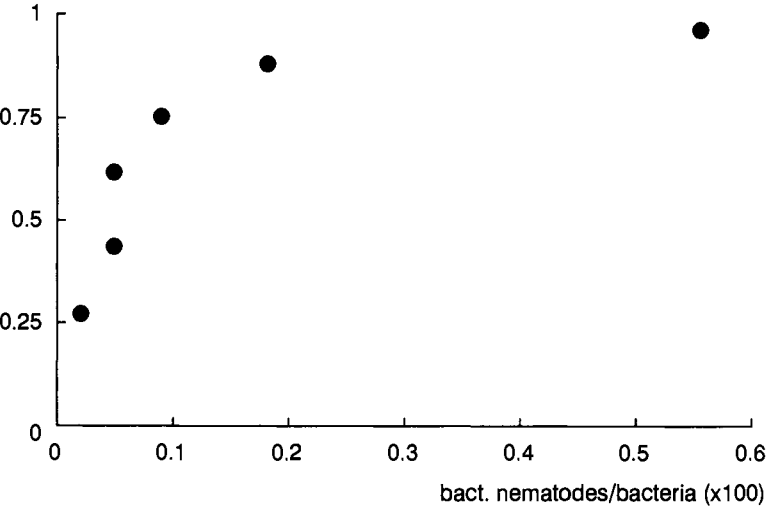


Fig. 7. Relation between the N mineralization rate ($\text{kg N ha}^{-1} \text{ day}^{-1}$) $\times 10^2$ per unit of biomass of bacteria (kg C ha^{-1}) and the biomass of bacterivorous nematodes (kg C ha^{-1}) per unit of biomass of bacteria (kg C ha^{-1}). (N mineralization/biomass bacteria is $0.96 (0.05) - 1.15 (0.17) \times 2.37 \times 10^{-7} (8.55 \times 10^{-7})^x$. x = biomass of bact. nematodes/biomass of bacteria; $r^2 = 0.95$).

N mineralization/biomass N ($\times 10^2$)

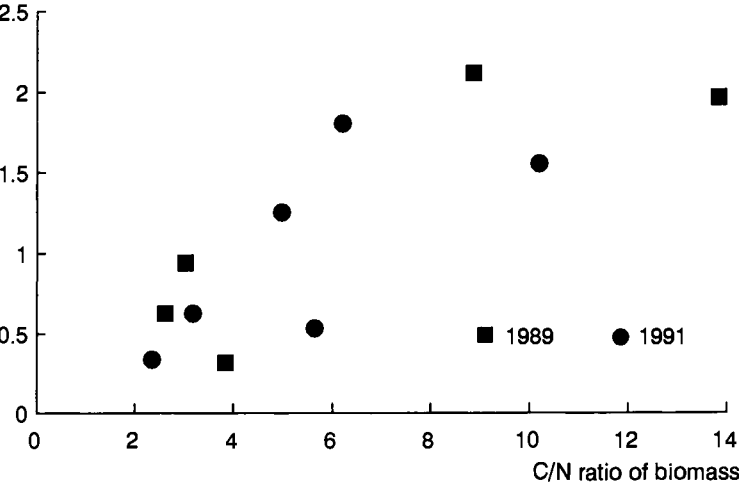


Fig. 8. Relation between the N mineralization rate ($\text{kg N ha}^{-1} \text{ day}^{-1}$) $\times 10^2$ per unit of microbial biomass N (kg N ha^{-1}) and the C/N ratio of the microbial biomass in 1989 and 1991. (N mineralization : biomass N is $0.17 (0.42) + 0.15 (0.04) \times \text{C/N microbial biomass}$; $r^2 = 0.67$.) () = standard error of difference.

microbial biomass was significantly higher ($p < 0.05$) in the sandy soils (6.2–13.8) than in the loams and clays (2.3–5.7).

Mineralization rates of C and N per unit of microbial biomass and bacterial biomass; relation with grazing intensity and C/N ratio of the biomass

To gain information about the activity of the bacterial and microbial population, the C mineralization rate ($\text{kg ha}^{-1} \text{ day}^{-1}$) was divided by the amount of bacterial and microbial biomass (kg C ha^{-1}). The ratios for the bacterial biomass ranged from 6.8 to 11.8 (Table 8). There were no differences between sandy soils, and loams and clays. The ratios for the microbial biomass ranged from 1.5 to 5.8 (Table 8). Again no differences between soil types were observed.

The N mineralization rate ($\text{kg ha}^{-1} \text{ day}^{-1}$) per amount of bacterial biomass C (kg ha^{-1}) was significantly ($p < 0.05$) higher in sandy soils than in loams and clays (Table 8). This was also the case for the N mineralization rate ($\text{kg ha}^{-1} \text{ day}^{-1}$) per amount of microbial N (kg ha^{-1} ; Table 8).

A positive correlation was observed between the N mineralization rate per biomass of bacteria and the grazing intensity of bacterivorous nematodes on bacteria (Fig. 7). Such a correlation was not found for the amoebae and flagellates. There was a positive correlation between the N mineralization rate per unit of microbial biomass and the C/N ratio of the microbial biomass ($R^2 = 0.67$; Fig. 8). Such correlations were not observed for C.

DISCUSSION

General

In this study we tried to determine whether sandy soils, loams and clays differ in C and N mineralization rates and whether observed differences are caused by differences in soil structural and biological characteristics. We investigated soils that had been used as intensively managed grassland for a long time. It may be assumed that the amount and quality of organic residues annually incorporated into the soil were similar and were not confounded with the effects of different textures and structural and biological characteristics.

The loams and clays were found to have a higher organic N content than the sandy soils. The only exception is the soil from Swifterbant. This location is a young sedimentary calcareous loam reclaimed from the sea only 30 years ago. At this location a large buildup of soil organic N is measured (Hassink and Neeteson, 1991). As the difference in organic C content between the soil types is much smaller than the difference in organic N content, the C/N ratio of the organic matter is considerably higher in the sandy soils than in the loams and clays (Table 1).

Generally, it is found that both the organic C and N contents are higher in soils with a higher clay content (Jenkinson, 1988; Spain, 1990). According to Jenkinson (1988) the C/N ratio of the soil organic matter is relatively constant for different soils under a wide range of management conditions. This is not in agreement with our observations.

The percentage of organic N mineralized during incubation was higher in sandy soils than in loams and clays (Table 2). This is in agreement with earlier observations (Catroux et al., 1987; Hassink et al., 1990). The percentage of organic C that mineralized, however, was not different for the soil types. This seems to contradict results of earlier experiments in which the decomposition of plant material was often found to be more rapid in sandy soils than in clays (Sørensen, 1975; Jenkinson, 1977; Ladd et al., 1990). In a recent study by Gregorich et al. (1991), however, no effect of soil texture was observed. In these experiments the decomposition of freshly added material was studied. Soil texture may have a different effect on the decomposition rate of such materials than on soil organic matter.

As the amount and quality of crop residues returned to the soil are similar in the soils studied, it may be assumed that in loams and clays relatively more N is retained in the soil than C.

Physical protection of organic N

We hypothesized that differences in N mineralization rate between soil types could be caused by differences in soil structural and biological characteristics. We assumed that in clay soils more organic N was physically protected against decomposition than in sandy soils. It may be concluded from our results that the mechanism of physical protection is different for different soil types. In sandy soils fine sieving did not increase the N mineralization rate. This suggests that no organic N was physically protected in small pores. The N content of the clay fraction in sandy soil, however, had a much higher N content than the total soil. This suggests that in sandy soils organic N is physically protected by the mechanism of adsorption to clay minerals or encrustation by clay minerals (mechanism suggested by Tisdall and Oades, 1982; Fig. 1). In the clay soils the situation was the opposite. Here the increase in N mineralization after fine sieving was considerable, while the N content of the clay fraction was not higher than the N content of the total soil. Apparently in clay soils organic compounds are physically protected against decomposition mainly due to their location in small pores (mechanism suggested by Elliott and Coleman, 1988; Fig. 2), while the association of organic compounds with clay minerals is less important. In loams, both types of physical protection seem to be important. There was some increase in mineralization after fine sieving and the N content of the clay particles was somewhat higher than the N content of the total soil.

Apparently N is much better protected than C. In the sandy soils, where organic material is protected by association with clay particles, the C/N ratio of this protected organic material was much lower than the C/N ratio of the total soil. In the clays, the organic material associated with clay particles had the same C/N ratio as the rest of the organic matter. In the clay soils, where it is assumed that organic material is protected in small pores, N mineralization was increased more than C mineralization after destruction of the soil structure (Table 4). This indicates that the C/N ratio of the released material is lower than that of the total organic matter.

Acridine orange is used to stain nucleic acids of living and dead organisms, but it is also bound by other macromolecules such as glycosaminoglycans, galactosaminoglycans, liposomes, negative phospholipids and colloids (reviewed by Paul, 1982). DTAF is used to stain proteins (Blakeslee and Baines, 1976). In our study, plant residues could easily be recognized using acridine orange or DTAF. The soil particles $<2\mu\text{m}$ did not show any staining with acridine orange or DTAF. The lack of stainable macromolecules in this fraction may reflect a higher degree of decomposition of the organic matter in this fraction. This indicates that this clay-associated protected material has been present in the soil for quite some time. Its low C/N ratio, small size and undefined structure suggests that it consists of microbial decay products.

Biological characteristics

The biomasses of the different groups were similar to those found in an arable soil in the Netherlands (Brussaard et al., 1990). In the arable soil fungal biomass was also very low in comparison with the bacterial biomass (Brussaard et al., 1990). Besides differences in physical protection, we also observed differences in biological characteristics among soil types. The loams and clays had a higher bacterial biomass than the sandy soils. The biomass of bacteria was correlated with the volume of soil made up by the pores between 0.2 and $1.2\mu\text{m}$. The most numerous soil microorganisms have been found to have a diameter $<0.3\mu\text{m}$ (Bae et al., 1972; Lundgren, 1984; Foster, 1985). Since the ratio between the diameter of pores and the bacteria they contain is approximately 3:1 (Kilbertus, 1980), a large proportion of bacteria may be expected to occupy pores $<1.2\mu\text{m}$, especially if the grazing pressure keeps bacterial biomass low in larger pores. The biomass of nematodes was correlated with the volume of pores between 30–90 μm . This is in agreement with the results of Jones et al. (1969) that most nematodes have diameters between 15 and 60 μm .

We have shown that the grazing intensity on bacteria by bacterivorous nematodes was higher in sandy soils than in loams and clays. The data suggest that the grazing of bacteria by bacterivorous nematodes increased N mineralization rates (Fig. 7). In general, the effect of a grazer on N mineralization

is high when the C/N ratio of its food is low, its own C/N ratio is high and its production efficiency is low (Hunt et al., 1987). Nematodes have a relatively high C/N ratio (7–10) compared to bacteria and a low production efficiency (Hunt et al., 1987). This means that they may potentially increase N mineralization. The absence of a correlation between the grazing intensity of amoebae and flagellates and the mineralization per bacterium may have been caused by the fact that biomass of amoebae and flagellates fluctuates considerably in sandy soils (Hassink et al., 1993).

We also observed that the C/N ratio of the microbial biomass was higher in sandy soils than in loams and clays (Table 7). There was a positive correlation between the N mineralization per unit of microbial biomass N and the C/N ratio of the microbial biomass (Fig. 8). This is in line with the concepts on which food webs are based. When bacteria decompose organic matter, more inorganic N is released from the organic matter when the bacteria have a higher C/N ratio and thus a lower requirement for N. C mineralization is not affected by the C/N ratio of the bacteria (Hunt et al., 1987; De Ruiter et al., 1993). The cause of the higher C/N ratio of the microbes in the sandy soils is not clear. It is assumed that fungi have a higher C/N ratio than bacteria. The fraction of fungi in the microbial biomass, however, is very small in all soils. According to Tezuka (1990), the C/N ratio of the microbes is also influenced by the C/N ratio of their food. One may assume however, that the C/N ratio of the organic residues returned to the soil should have approximately the same C/N ratio since all soils in our experiments are intensively managed grasslands. According to Scholefield et al. (1991) the C/N ratio of 1 year old residues of dead plant material in intensively managed grassland soils is 12.5.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

Differences in pore size distribution between soil types affect the relative importance of different mechanisms of physical protection of organic matter. Pore size distribution also determines the grazing intensity on bacteria. The C/N ratio of the microbial biomass was lower in clays and loams than in sandy soils. We have no explanation for this difference.

At the moment it is not clear whether all factors are important or whether just one of these is the key factor that determines the N mineralization rate in grassland soils. The measurements described here should be tested on a wider range of soils, including soils with the same texture but with a different pore size distribution. This would show whether observed differences are related to pore size distribution or to clay content of the soil. Ultrastructural studies focusing on the exact location of organic matter and microbes are necessary to obtain additional evidence for our hypothesis. Simulation modelling should then indicate which of the observed differences are the key factors in determining the N mineralization rate.

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A method for the three-dimensional mapping of earthworm burrow systems

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ABSTRACT

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To study the spatial structure of earthworm burrow systems a field method was developed to map these systems three-dimensionally. The method is based on serial sectioning of the soil. An important feature of the method is the use of the orientation of the burrows in order to reduce the number of layers needed for a reliable reconstruction of the burrow system. The burrow system is reconstructed from the measured data by using search windows of which the position and size depend on the orientation of each single burrow. An example of the three-dimensional structure of an earthworm burrow system made by *Aporrectodea longa* and *A. caliginosa* is given.

INTRODUCTION

The influence of earthworm burrow systems on the soil air and soil water dynamics has been the topic of numerous studies, e.g., Bouma et al. (1982), Hoogerkamp et al. (1983), Zachmann et al. (1987) and Edwards et al. (1990). In most of these studies the earthworm burrow system is described only in terms of burrow numbers per unit area, sometimes measured at several depths. The rough effects the earthworm burrow system has on the soil air and soil water dynamics as through the magnification of the saturated hydraulic conductivity or a higher air permeability compared with absence of the burrow system, can be established this way. A more detailed analysis of the influence of the burrow system on the soil water and air economies requires that its three-dimensional structure, and so its topology is known.

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To achieve this it is necessary to map the burrow systems three-dimensionally. In this paper a field method for this mapping is presented. The method will be used in a future study on the influence of earthworm burrow systems on the soil water economy.

TECHNIQUES

The three-dimensional imaging techniques that could be used for the three-dimensional mapping of earthworm burrow systems can be subdivided into two main groups: destructive and non-destructive techniques. Use of a non-destructive technique in the most strict sense leaves the burrow system in place and undisturbed and should not alter the behaviour of the earthworm population.

The most promising non-destructive three-dimensional imaging techniques are, at this moment, computer-assisted tomography also called X-ray computed tomography and magnetic resonance imaging. The latter which is also known as MRI or NMR provides information about the nuclei of atoms (Pykett, 1982). A three-dimensional data set is created containing this information. The application of this technique in soil science is still in an experimental phase. Also the high costs prevented it from being used by us to study the structure of earthworm burrow systems.

The other technique mentioned, computer-assisted tomography, is based on the scanning of a body with X-rays in several "slices". A three-dimensional data set of the X-ray attenuation coefficients is created, consisting of elements with a volume of, for instance, 2 mm³. Air-filled pores have an attenuation which is considerably smaller than that of the surrounding matrix and so these pores can be extracted from the data set. Computer-assisted tomography has been used to study soil density (Jenssen and Heyerdahl, 1988), soil macropores of different origin (Warner and Nieber, 1988) and earthworm burrow systems (Joschko et al., 1991).

Computer-assisted tomography has at the moment one main disadvantage, which is the very high expenses needed to obtain the data. Due to these costs it was not used by us to study the three-dimensional structure of earthworm burrow systems.

The destructive techniques that can be used to determine the three-dimensional structure form two groups, excavation or sectioning techniques and casting techniques.

Casting techniques are based on filling earthworm or other burrows with a liquid that hardens after some time. The viscosity of the liquid determines the smallest pore size into which it can enter. Plaster of Paris (Bouma et al., 1982; Joschko, 1989), latex (Garner, 1953), polyester resin (Rogaar, 1974) and polyurethane foam (Kobel-Lamparski and Lamparski, 1987) have been used for this purpose. After the hardening of the liquid the soil around the

cast is removed. Orientation, position and for instance diameter or volume can be measured directly at the casts or from depictions of them.

One important method which uses sections through a structure to gain three-dimensional information from this structure is stereology. Stereology is defined by Weibel (1979, p. 1) as "a body of mathematical methods relating three-dimensional parameters defining the structure to two-dimensional measurements obtainable on sections of the structure". Stereology has been applied to tubular structures (Baddeley and Averbach, 1983; Cruz-Orive et al., 1985) resulting in estimations of the volume, surface and length densities of the structures. By using directional statistics Cruz-Orive et al. (1985) also gained information about the distribution of directions of an anisotropic tubular structure. Information about the degree of connectivity or continuity of the structure cannot be obtained by these stereological methods making a truthful reconstruction of the structure impossible. Other sectioning or excavation techniques can be used to assess this reconstruction needed to model for instance the influence of a burrow system on the flow of water inside the soil.

These techniques can be burrow oriented or layer oriented. In the burrow-oriented approach each single burrow is exposed bit by bit and at several points its coordinates are measured. The layer-oriented approach uses excavation in several parallel layers, of the exposed burrows the coordinates are measured. The direction of excavation can be horizontal (Kretzschmar, 1978) or vertical (Ehlers, 1973; Edwards et al., 1988). The mapping of the burrow system can be direct by recording the coordinates in situ or indirect by photographing or making drawings at first and measure the coordinates from these representations. Kretzschmar (1988) made a three-dimensional reconstruction of the earthworm burrow system by using a column of soil of $10 \times 10 \times 200$ cm and destroying it little by little to expose and measure the coordinates of each burrow. Edwards et al. (1988) photographed horizontal surfaces with exposed earthworm burrows at four different depths and used image analysis techniques to recognize, count and describe the burrows. Ehlers (1973) used more closely spaced (between 2 and 20 cm) horizontal surfaces and counted the burrows which were divided into three diameter classes.

The reconstruction of the burrow system from layer by layer excavation is based on serial or sequential sectioning, a technique mainly used for studying microstructures (DeHoff et al., 1972; Yanuka et al., 1984). Serial sectioning requires a series of closely spaced parallel sections through a structure, in order to reconstruct it three-dimensionally. The spacing between two successive layers depends on the scale of the structure to be studied (DeHoff et al., 1972). This scale may be described by the mean intercept of the phase of interest, λ , in the case of an earthworm burrow system λ can be translated into the mean width of intercepted burrows on a layer. DeHoff et al. (1972) recommend a spacing of $1/3$ to $1/10 \lambda$.

A preliminary test using several excavating and casting techniques in the field showed that these latter techniques (polyester resin and gypsum) do not give a reliable reproduction of the burrow system. The buildup of pressure inside a burrow by the invading liquid may cause a partial filling of the burrow. Using liquids with lower viscosity results in the enclosure of soil particles or aggregates thus obscuring burrows. Another disadvantage of casting is that it only reveals the burrows that are open to the surface, obstructed burrows or burrows with no contact with the surface cannot be found with this technique.

Excavating techniques combined with photography of the burrows showed that the burrows have to be marked before photographing them in order to recognize them properly on the images. Stereo photography does not request the measurement of the depth position(s) of the layers but this technique appeared to be too expensive. Among the excavating techniques we found the layer by layer approach combined with direct mapping to be the most practical one. Especially when working with large volumes and when collapsing of the soil column is probable. The method and procedure together with some of the first results are presented in the following.

METHOD

Introduction

Our method to map earthworm burrow systems three-dimensionally is based on serial sectioning. The recommendations of DeHoff et al. (1972) refer to isotropic networks, for oriented systems, however, a wider spacing can be used when the sections or layers are perpendicular to the orientation of the system. The reason for this is that horizontal sections have a much greater chance of hitting a vertical element than vertical sections (Ringrose-Voase and Nortcliff, 1987). Translating DeHoff's rule of thumb on the spacing of sections ($1/3-1/10 \lambda$) to an earthworm burrow system with 3.5 mm as mean intercept results in a spacing of 1.2 to 0.35 mm. This would result in an impracticable number of layers for a 1 m high soil column. However, because of the vertical orientation of the earthworm burrow systems at the study site, a wider spacing could be used. Further increase of the spacing is obtained by using the orientation of each burrow in the reconstruction process thus greatly reducing the area in which to search for a matching burrow (see reconstruction procedure). Wendt and Larink (1990) used a spacing of 1 cm to reconstruct earthworm burrows which had been formed in artificial soil cores. A spacing of the same magnitude has been used for our measurements.

Field site

In 1957 the polder Eastern Flevoland was reclaimed from the IJssel Lake, in the new soil no earthworms were present. In this polder earthworms were inoculated in 1983 at the Ir. A.P. Minderhoudhoeve, an experimental farm of the Wageningen Agricultural University, to study the effect of earthworm activity on the production rate of pastures. The earthworms were inoculated in two rows, separated 12 m apart, at one side of the plot. Individuals of the following species were placed under the turf: *Aporrectodea longa*, *A. caliginosa*, *Lumbricus terrestris* and *L. rubellus*.

Mapping of the burrow systems took place in August and September 1991 in this inoculated permanent pasture used for grazing and mowing situated at the Ir. A.P. Minderhoudhoeve. The soil is a Calcaric Fluvisol with a loam texture. In the summer of 1991 the earthworms had spread ~ 42.5 m into the plot. The burrow systems were mapped in four pits located between the point of inoculation and the point that had just been reached by the earthworms.

Field procedure

At one side of a large pit of circa $2 \times 2 \times 1$ m a square soil column of $\sim 0.7 \times 0.7$ m was maintained. Previously to the digging the surface of the column had been irrigated with a methylene blue solution to stain burrows open to the surface. The column was excavated in successive horizontal layers ranging in thickness from 1 to 3 cm. After excavating of a surface with a sharpened stopping-knife the surface was brushed clean. Occasionally the soil was slightly smeared, causing the closure of burrows, or soil material was pushed into the burrows. By careful examination of the surface such burrows were spotted and re-opened. Burrows that were naturally filled, e.g., by earthworm casts were not opened. On each exposed surface (measuring area 0.5×0.5 m) the coordinates of the open burrows equal to or greater than 2 mm were measured directly by a manually operated measuring apparatus (Fig. 1). Besides the coordinates the diameter, the presence of methylene blue, and the orientation were recorded. The coordinate and diameter were measured to the nearest millimetre. The orientation was described in terms of dip and strike (see Fig. 2), the dip being the angle with the horizontal plane was, following Kretzschmar (1982), divided into three classes: 0–30, 31–60 and 61–90°. The strike, the angle with the y-axis was recorded in eight classes of 45° (north, northeast, etc.) a ninth class was added which represents burrows with a pure vertical orientation, thus having no strike. Dip and strike were estimated visually, in doubtful cases a compass or clinometer was used. Of horizontal burrow parts the end points and intermediate points where the burrow changed its direction were used. Particularities as, e.g., branching of

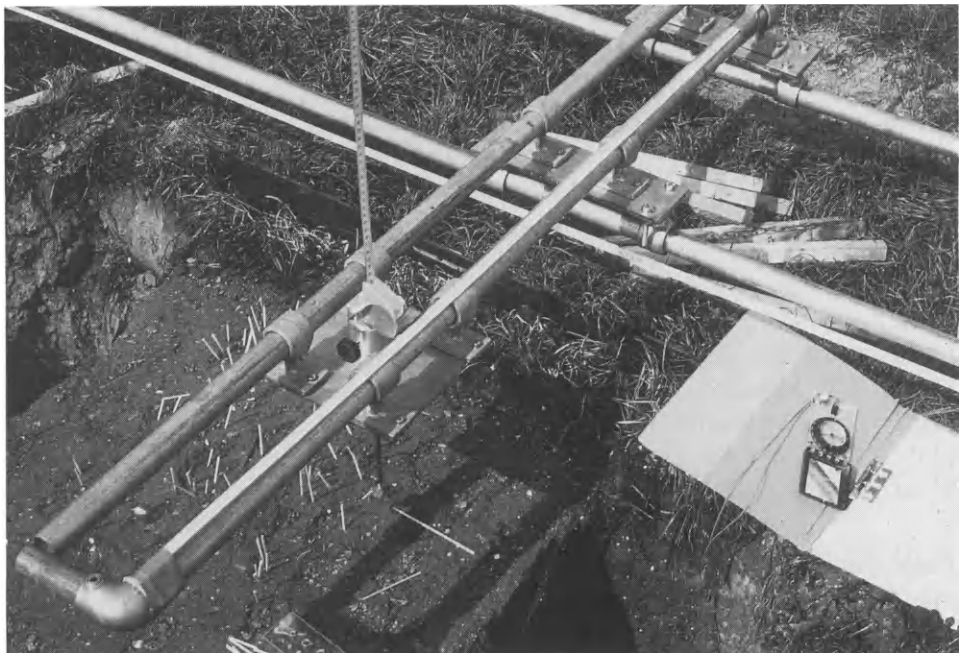


Fig. 1. Measuring apparatus with in background support for x -position runner and from back to front x -position runner with mounted y - and z -position runners. The position is read from the attached measuring tapes.

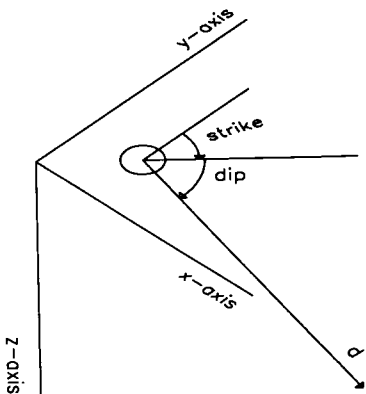


Fig. 2. Dip and strike for a burrow with direction d . The burrow is depicted as a horizontal circle.

burrows, partial infilling, presence of earthworms, were noted and were used in the manual reconstruction process.

Reconstruction procedure

The reconstruction is based on the projection of the boundaries of the dip and strike classes of a burrow on layer L onto the layer $L + 1$, starting with the top layer (Fig. 3). The area on layer $L + 1$ in which to find a matching burrow for a certain burrow at layer L is called the confidence area. The size and shape of this area depend on the orientation of the burrow at layer L and of Δz_L , which is the distance between layers L and $L + 1$. Estimation errors in the dip and strike occurring during the measurement or estimation are assumed to be $\pm 5^\circ$ and are accounted for in the confidence area. The confidence area (Fig. 3) is created by adding to the sector that is formed by projecting the boundaries of the strike and dip classes on layer $L + 1$ the projection of a cone with a width 10° . This cone reflects the estimation errors of $\pm 5^\circ$. For a burrow having a northeastern strike, that is between 22.5 and 67.5° , the confidence area lies between 17.5 and 72.5° . The boundaries of the dip class for a burrow with a dip of 31 – 60° become, by adding the uncertainty of $\pm 5^\circ$, 26 and 65° . Burrows that lie inside the confidence area and have, within a range of minus or plus 2 mm, the same diameter are called matching burrows (Fig. 4).

The search algorithm (Fig. 5) was implemented in the dBASE * command language. For reasons of programming efficiency the confidence areas are translated into square search windows. The size, shape, and the position of the midpoints $(x, y, z)'$ of these windows (Fig. 4) are calculated from the orientation of the burrow at layer L and from Δz_L (see below). The midpoints

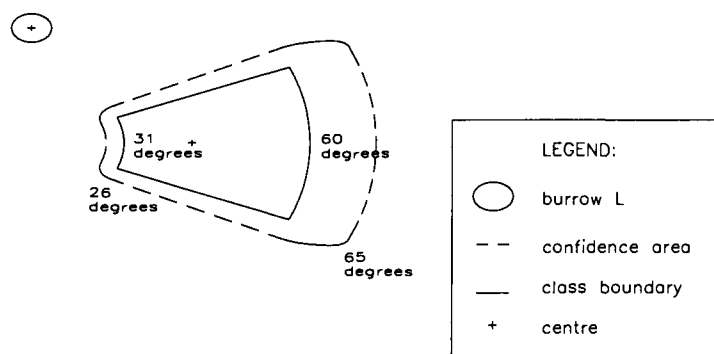


Fig. 3. The confidence area is based on the boundaries of the strike and dip class of a burrow. The direction of view makes an angle of 45° with the horizontal plane.

*dBASE is a trademark of Ashton-Tate Inc.

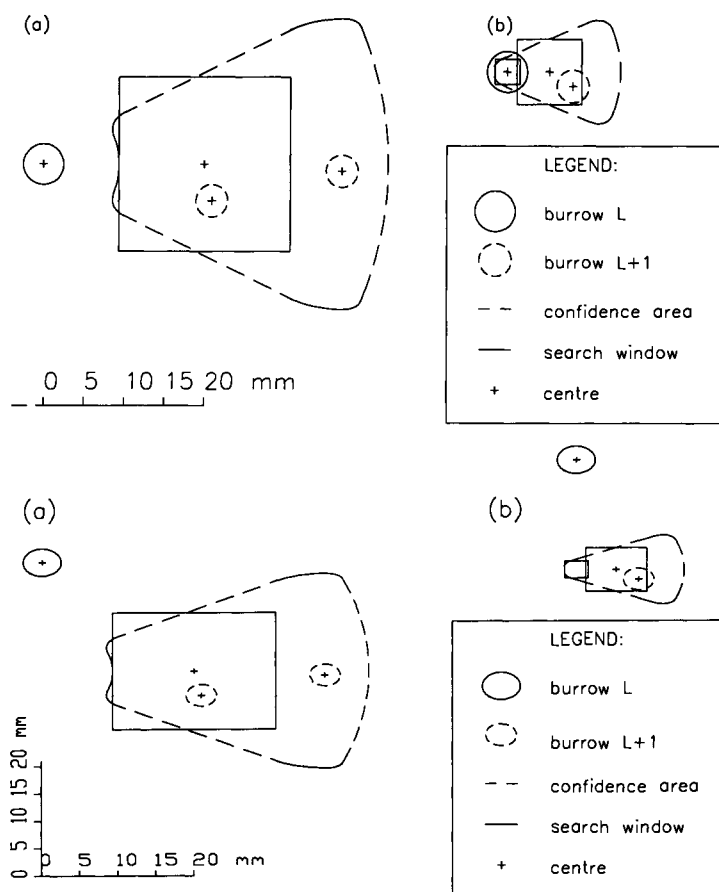


Fig. 4. (Top) Search window(s) and confidence area for a burrow with (a) a dip of 31–60° and (b) a dip of 61–90°. The direction of view is perpendicular to the horizontal plane. (Bottom) Search window(s) and confidence area for a burrow with (a) a dip of 31–60° and (b) a dip of 61–90°. The direction of view makes an angle of 45° with the horizontal plane.

of the search windows lay on the bisecting lines of each dip and strike class reflecting an estimation of the mean orientation per class.

In the case of the dip being close to 90° too few connections can be made with the window based on the bisecting lines (window 2), therefore a smaller window (window 1) is added in the search procedure. This window is based on the projected area of a cone with a width of 10°, reflecting the confidence area at a dip of 90°. The first search is made inside this small square window (window 1). If no connections can be made window 2 is used for searching.

The search window used for dips of 31–60° has an area which is 50% of that of the confidence area. In case of the dip being greater than 60° the area of the combined windows 1 and 2 is about 60% of that of the confidence area.

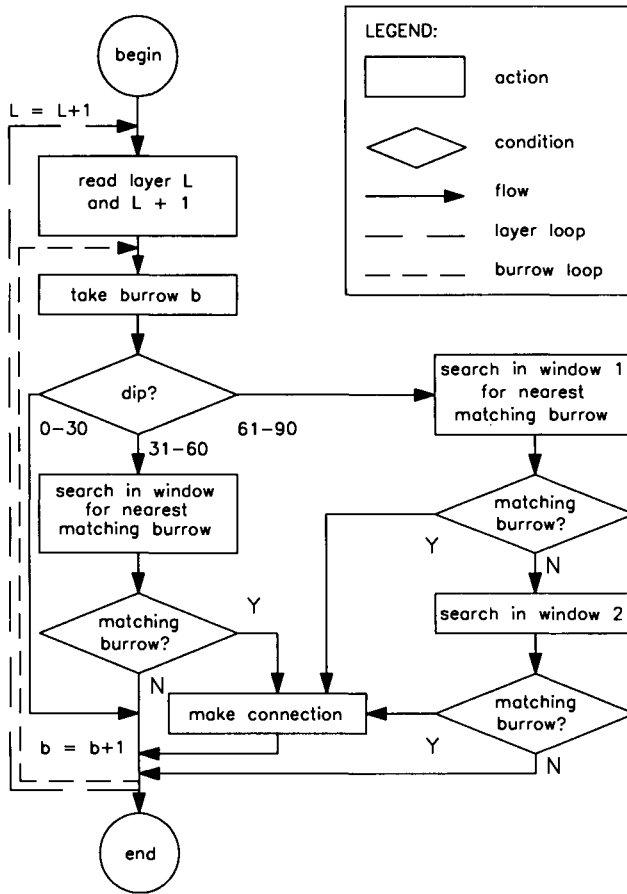


Fig. 5. Flow diagram of the search algorithm.

Less than 10% of the area of the squares is located outside the confidence area.

$dip = 61-90^\circ$

Search window 1:

$$\text{width} = 0.154 \times \Delta z_L \quad (1)$$

$$x' = x \quad (2)$$

$$y' = y \quad (3)$$

$$z' = z + \Delta z_L \quad (4)$$

Search window 2:

$$\text{width} = 0.4 \times \Delta z_L \quad (5)$$

$$x' = x + (\tan(15) \times \cos(\text{strike}) \times \Delta z_L) \quad (6)$$

$$y' = y + (\tan(15) \times \sin(\text{strike}) \times \Delta z_L) \quad (7)$$

$$z' = z + \Delta z_L \quad (8)$$

$$\text{Dip} = 31-60^\circ$$

$$\text{width} = 1.07 \times \Delta z_L \quad (9)$$

$$x' = x + (\tan(45) \times \cos(\text{strike}) \times \Delta z_L) \quad (10)$$

$$y' = y + (\tan(45) \times \sin(\text{strike}) \times \Delta z_L) \quad (11)$$

$$z' = z + \Delta z_L \quad (12)$$

The dBASE programme only uses burrows with a dip greater than 30° . Automatic connections of horizontally oriented burrows are prone to errors in these connections because the search window can become very large.

After running of the dBASE programme the output file with data of the burrows and the connections between them was read into AutoCAD*, a CAD package. The burrows were displayed on screen as circles with diameters as measured. The connections between burrows are displayed as lines running between the centres of the burrows (Fig. 6). All the unconnected burrows were checked manually for the possibility to connect them with a burrow lying outside the window but inside the confidence area on layer $L + 1$. If this proved to be possible a connection was made manually in AutoCAD. If not, the search procedure was repeated manually using the layer $L + 2$. The reason for using this layer is that in the field sometimes open burrows, stained by methylene blue, were missed and re-appeared at the next layer.

The automatic procedure only creates non-branching burrows. Branching burrows were created manually when these had been observed in the field.

RESULTS

The first results of the method presented here are given as an example and do not represent a full analysis of the three-dimensional structure and topology of earthworm burrow systems. The results are based on the burrow system at 40 m from the inoculation site having an age of ~ 1 year. This burrow system is created by *Aporrectodea longa* and *A. caliginosa* with, respectively, abundances of 141.1 and 16.7 g/m². To map the burrow system 41 layers were used, the mean distance between them was for the first ten layers 1.2 cm

*AutoCAD is a trademark of Autodesk Inc.

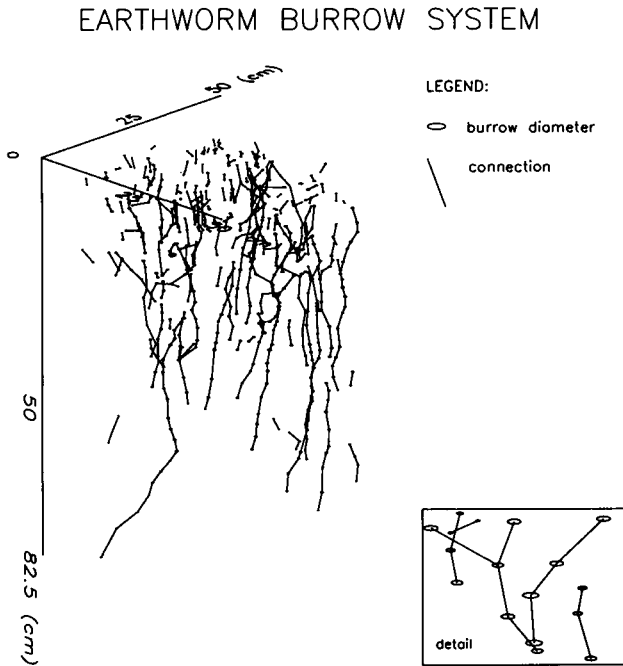


Fig. 6. Three-dimensional representation of earthworm burrow system. Only connected burrows are shown.

and for the total set 2.0 cm. The system is characterized by mainly vertically oriented burrows (Fig. 6). Only a small number of burrows have first-order branches which are horizontal or directed upwards.

Of the burrows 95% could be connected with those on the layer below which means that they were at least continuous over the distance. Many of the deeper burrows are stained by methylene blue and are thus connected with the surface. However, it was not possible to connect all of them directly to the surface (see Discussion).

DISCUSSION

A mean distance of 2 cm between horizontal layers used for serial sectioning appears to be applicable for describing vertically orientated earthworm burrow systems when used in combination with the measured orientation. This spacing far exceeds the one following from the rule of thumb on serial sectioning for isotropic systems (DeHoff et al., 1972), which for the here used earthworm burrow system calls for a spacing of 0.35 to 1.2 mm.

Due to the crumbly structure and the abundance of grass roots the preparation of the top layers is more difficult than for the deeper layers resulting in

missed burrows. Probably these missed burrows are the cause of the inability to connect many of the more continuous burrows directly to the surface layers. Until now no reliable estimation can be made of the percentage of missed burrows in the top layers.

The results show that the developed apparatus and the described mapping method are suitable for assessment of the three-dimensional structure and topology of earthworm burrow systems in the field. A disadvantage is, however, that the method requires a fair amount of labour.

The use of the method could be extended to the study of other three-dimensional soil macrostructures as for instance cracks.

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Water movement, oxygen supply and biological processes on the aggregate scale

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ABSTRACT

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An experimental respirometer system and a theoretical simulation model were developed to study the dynamics of water movement, oxygen supply and biological processes on the aggregate scale in unsaturated soil.

The experimental system enables one to measure simultaneously the distribution of water, oxygen, and chemical substances as a function of space and time in an unsaturated, artificially made, homogeneous, cylindrical aggregate and the changes in atmospheric composition as a function of time in the chamber that contains the aggregate. Non-destructive measurements during an experiment involve gamma ray attenuation, gas chromatography and polarography. Destructive measurements are performed at the end of an experiment in the form of chemical analyses of soil.

The theoretical model enables one to calculate simultaneously the distribution of water, bacteria, gases (e.g. oxygen, carbon dioxide and molecular nitrogen), absolute soil atmospheric pressure and solutes (e.g. nitrate and glucose) as a function of space and time for exactly the same cylindrical geometry as in the experimental set up. Also, the changes in atmospheric composition as a function of time in the chamber that contains the aggregate are calculated. Except for water transport, all processes are caused by microbial activity, since roots are absent in the aggregate.

The respirometer system was designed to generate coherent data sets to evaluate the simulation model, while the model was needed to more completely interpret the data obtained from the experiments.

The reported experiment shows that hysteresis in the soil water characteristic strongly affects the water distribution in the aggregate. As a result, the oxygen supply to the interior of the aggregate is decreased to such an extent, that anaerobiosis is maintained there after the oxygen is consumed.

The respiratory quotient (RQ) was about one in the experiment, although one would expect larger values in partially anaerobic soil. Therefore, the RQ does not seem a sensitive measure to decide whether a soil is partially anaerobic. Nevertheless, the consumption rate of oxygen and the production rate of carbon dioxide compared well with field data. This is the result of the pretreatment of the soil, which aimed at avoiding the flush of microbial activity upon wetting.

The simulated results showed a satisfactory agreement with the experimental data: part of the ex-

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perimental results could be described quantitatively, whereas other data that deviated from the experimental data could be understood by studying the dynamic behaviour of the model. Hysteresis in the soil water retention curve resulted in low values of the gas-filled porosities in the outer shell of the partially wetted aggregate, permitting gaseous exchange only through the water phase of soil. As a result anaerobiosis occurred. The model is very sensitive for the so-called critical gas filled porosity below which no gas-continuous pores exists. The simulated respiratory quotient was seen to be strongly affected by transport processes. Therefore, also on theoretical grounds it was concluded that RQ is not a sensitive indicator of partial anaerobiosis in soil.

The objective of this paper is to describe both the experimental respirometer system and the theoretical simulation model, and to report some of the measurements.

INTRODUCTION

In aggregated soils, intra- (micro) and interaggregate (macro) pores may be found. Both types of pores determine the transport properties of the soil for water, solutes and gaseous substances. As a result they determine the biological activity of the soil. The macro pores are a major determinant in the transport of substances through the soil from and to the atmosphere, whereas the micro pores are important in the transport of substances from the larger pores in the soil into and out of the aggregates, structural elements, or more generally, the denser structures in the soil.

Soils accommodate roots and fauna: the roots and the larger animals may contribute to the soil's macro pore system, while the microorganisms usually are fixed in their environment and act as small factories that transform substances passing by. The roots and the larger animals need oxygen for their life processes. Roots may have aerenchyma enabling them to live in water-saturated soil where oxygen is absent. The larger animals are bound to live above the ground water table in the macro pores and the cracks of the soil, which are partly created by them. The microorganisms, however, may be highly adapted to their environment: different species may live under strict aerobic circumstances, or under strict anaerobic circumstances, while some species may live in both of these environments.

In aggregated unsaturated soils, oxygen consumption potentially leading to anaerobiosis is mainly confined to the aggregates. Figure 1 depicts some schematic oxygen and water distributions as expected in field aggregates under the assumption of a homogeneous distribution of bacteria and organic compounds. When the oxygen consumption rate does not exceed the oxygen supply rate, anoxic conditions will not develop, and equimolar respiration occurs, as indicated by arrows (Fig. 1a). Just after rainfall, mainly the outer shell of an aggregate will be wetted (Leffelaar, 1979). The oxygen diffusion rate in the outer shell is then seriously impeded, and when the oxygen consumption rate exceeds the oxygen supply rate, anoxic conditions arise locally (Fig. 1b). In the center of the aggregate respiration continues to take place until the oxygen from the enclosed air has been consumed. Then most of the aggregate volume is anaerobic, and anaerobic processes can occur very near

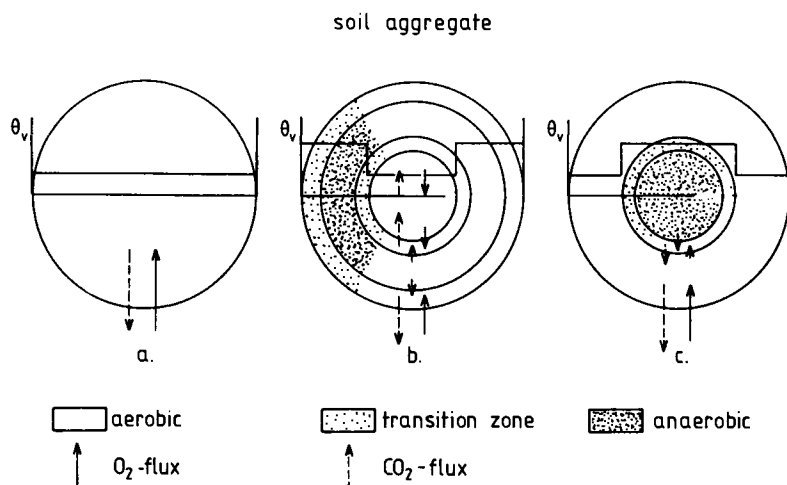


Fig. 1. Schematic water and oxygen distributions in soil aggregates: (a) in a dry period, (b) just after a rain shower, (c) some time after rainfall. Lengths of arrows indicate relative magnitudes of molar source or sink terms.

to aerobic processes in the outer shell. Subsequent redistribution of water may result in a decrease of the anaerobic aggregate volume, and hence of anaerobic processes, when the water content in the wetted shell becomes low enough to give continuous gas-filled pores permitting rapid oxygen diffusion into the aggregate. The distribution of oxygen in Fig. 1c will be found in initially water-saturated aggregates that are drying. Upon further drying their oxygen distributions will adjust to that of Fig. 1a.

These complicated dynamic interactions between biological and physical processes determining the oxygen status of soil and hence the occurrence of aerobic and anaerobic processes at the micro scale were studied both experimentally and theoretically. For the experimental study a respirometer system was developed that enables us to generate coherent data sets on water and gas movement and biological processes occurring on the micro scale. For the theoretical study, an explanatory simulation model was developed that includes descriptions of these processes. In fact the experimental system was developed to evaluate the theoretical model, while the theoretical model was needed for a more complete interpretation of the measured data.

The objective of the present paper is to describe the experimental respirometer system, to discuss the explanatory simulation model, and to compare the results of an experiment with those of the model.

MATERIALS AND METHODS

The experimental soil aggregate on which the measurements of the spatial distribution of oxygen, water, and decomposable organic compounds as a

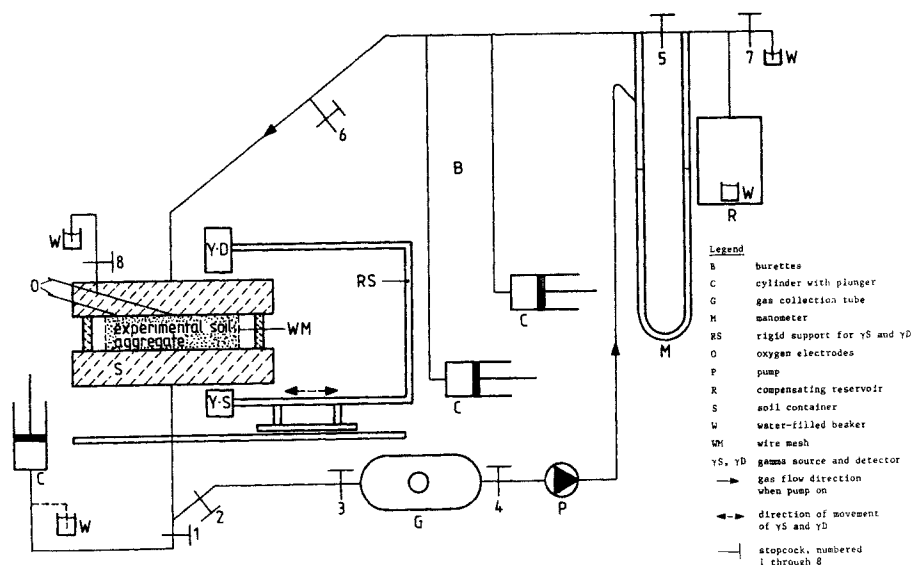


Fig. 2. Diagram of respirometer system showing soil container, gas collection circuit (stopcocks 1 through 7 and attached devices), oxygen electrodes and gamma ray attenuation apparatus with rigid support.

function of time are performed, is a short cylinder of homogeneously packed soil material in which transport processes are radial. This model representation of a soil aggregate originates directly from Fig. 1. A scheme of the experimental installation is given in Fig. 2.

Soil

A sandy loam soil from Lelystad was taken from the surface 0–25 cm layer and stored under field moist conditions. Some characteristics are: pH (measured in 4 gram of soil suspended in 10 ml of liquid) in H_2O and KCl: 7.8 and 7.4, respectively; $CaCO_3$ (Scheibler's method described by Allison and Moodie, 1965): 6%; organic carbon (Mebius, 1960): 1.2%; total nitrogen (Novozamsky et al., 1984): 0.09%; CEC- $BaCl_2$ (Bascomb, 1964): 10.1 cmol(+) per kg of soil; and soil texture (Pipette method described by Day, 1965) 2 μm , 20 μm and 50 μm : 13, 27 and 32%, respectively. The soil is air-dried, pulverized to pass a 0.5 mm sieve, directly remoistened to about 0.1–0.15 $g\ g^{-1}$ and left in a closed container for at least 6 weeks to let the metabolism of microorganisms recover (Birch, 1958, 1959; Fillery, 1983). Remoistening is attained by adding an appropriate amount of ice scrapings to soil and mixing in a mortar with a pestle. After installing the experimental aggregate in the measuring system, it is left alone for at least a week to let the me-

tabolism of the microorganisms recover. Then, an experiment is started. This pretreatment of the soil effectively prevented the flush of microbial activity upon wetting.

Measuring system

The measuring system (Fig. 2) was installed in a constant temperature room ($22.7 \pm 1.5^\circ\text{C}$) and is composed of four subsystems:

(1) Container (S) which holds the experimental soil aggregate and the oxygen electrodes.

(2) A closed gas collection circuit (stopcocks 1 through 7 and attached devices) which serves three purposes:

- it contains enough oxygen to meet the respiratory demand of the microorganisms in the soil aggregate during one experiment.
- it functions as volumeter to determine net changes in the total volume of gas resulting from respiration.
- it is used to withdraw gas samples to assess gaseous composition by gas chromatography.

(3) Two polarographic Clark-type oxygen electrodes (Kreuzer et al., 1980) to measure oxygen pressure in the center and 4 cm from the center of the experimental soil aggregate.

(4) A gamma ray (60 keV radiation from ^{241}Am) attenuation apparatus (resolution of 1 mm) (De Swart and Groenevelt, 1971) to measure the radial distribution of soil water content.

The soil sample of 10 cm diameter and a height of 2.5 cm has a density of about 1.4 g cm^{-3} , and is radially supported by a wire mesh of more than 70% porosity. The compensation reservoir, R, serves to minimize the influence of barometric pressure changes. Pressure differences between the gas circuit and the compensation reservoir are eliminated by adjusting the water levels in the burettes B. The change in burette volume is a measure for the net change in gas volume due to respiration in the soil aggregate. The gases in the gas collection circuit are thoroughly mixed by pump P, before a gas sample is taken from gas collection tube G. Gas samples of 0.1 ml were analysed on a Packard–Becker 427 gas chromatograph using Porapak Q and Molecular Sieve 5A columns in series and a thermal conductivity detector. The Clark-type electrodes were selected for their small oxygen consumption, compared with that of soil, and their good stability and linearity over 1 to 7 days of use.

In a typical experiment, the effect of a rain shower on the aggregate was mimicked by wetting the aggregate with a small amount of solution containing potassium nitrate and organic matter (glucose) equivalent to about 65 kg ha^{-1} (N and C). Then, the redistribution of water and the dynamics of gases inside and outside the aggregate were recorded. At the end of the experiment the aggregate was partitioned into five concentric layers of 1 cm thickness

each, in which gravimetric moisture content and chemical analysis (no data reported here) were determined.

Reliability of the data so obtained was assessed by calculating the propagation of the errors in the measured quantities on the final results by procedures outlined by Berendts et al. (1973). To this purpose, the errors in the measured quantities were evaluated from knowledge about the measuring instruments.

Full technical details, comments on procedures and calculations to obtain the data are given in the work by Leffelaar (1986).

SIMULATION MODEL COMPRISING SUBMODELS FOR RESPIRATION, WATER, SOLUTES, AND GASES

An explanatory simulation model was developed for the same geometry as depicted in Fig. 2 to calculate the distribution of the relevant state variables, here bacteria, water, solutes, and gases, as a function of space and time in an unsaturated aggregate in which transport processes are radial. Also the changes in atmospheric composition as a function of time, in the chamber that contains the aggregate, are calculated.

The simulation model comprises four submodels: one for the biological process of respiration and denitrification, and three for the transport processes of water, solutes (decomposable organic compounds, i.e. glucose), and gases (e.g. oxygen, carbon dioxide and molecular nitrogen), respectively. To calculate the spatial distribution of the various state variables, the system was divided into a number of concentric layers (Frissel and Reiniger, 1974; De Wit and van Keulen, 1975). The interactions between the system components are reflected in the interactions between the four submodels. This may be demonstrated by the equation of continuity, eq. (1), that is solved for each mobile substance in each layer:

$$\frac{\partial C_i}{\partial t} = -\frac{\partial J_i}{\partial x} + P_i \quad (1)$$

Symbols are defined in the Appendix. For instance, the respiration submodel calculates different gas production and consumption rates (P_i) in adjacent aggregate layers, because the bacteria in these layers are subjected to different environmental conditions. Different production and consumption rates result in gradients in the concentration of substance i . When it concerns a gaseous substance, in principle also gradients in absolute pressure result. The flux J_i includes the diffusive, dispersive and mass flow components. Integration of eq. (1) with respect to time will give the time course of substance i in a layer as a result of the interactions of microbial activity and physical transport processes. When a substance is inert, e.g. molecular nitrogen, or

immobile, e.g. biomass, the production term or the flux term in eq. (1) equals zero.

To develop the submodels, numerous assumptions had to be made. Many of these assumptions have been described elsewhere: the respiration and denitrification model by Leffelaar and Wessel (1988); the water flow model by Dane and Wierenga (1975); the solute transport model by Bolt (1979); and the gas transport model by Leffelaar (1987, 1988). Therefore, these models and their assumptions are merely summarized below. However, the interactions between the submodels of the water with the gases, and with the gas production and consumption terms due to respiration, imposed difficulties that are not normally envisaged when such models are developed or used separately. Therefore, these are mentioned in somewhat more detail.

Respiration submodel

In the submodel describing respiration, the growth of two groups of heterotrophic strict aerobic bacteria, of which a part is capable to grow under anaerobic conditions with nitrate as electron acceptor, was calculated by a first order rate equation. (The model was originally developed to simulate denitrification in the anaerobic part of the aggregate.) The relative growth rate was described by a double Monod equation consisting of rate limiting factors for carbon substrate and oxygen electron acceptor. Changes in the amounts of oxygen and glucose carbon were described by Pirt's equation (Pirt, 1975), where growth yields and maintenance coefficients of the bacteria are distinguished. The release of carbon dioxide was calculated from the difference of the consumption rate of glucose and the rate of carbon used to form biomass. Thus it was assumed that complete oxidation of the organic matter occurred. The microorganisms were assumed to be immobile (Woldendorp, 1981) and active. The environmental conditions that affect growth and respiration, i.e. organic matter content and electron acceptor, were assumed to equal the concentrations of these substances in the water phase of the soil. Soil water content will thus have a profound influence on these concentrations. First, different concentrations directly affect relative growth rates through the Monod equation. Second, low water contents will decrease the rate of diffusion over short distances (microdiffusion) of nutrients to bacterial colonies or cells. The submodel was developed for a homogeneous soil layer. In the simulation model each concentric layer of the aggregate was also assumed homogeneous. Therefore, the respiration submodel could simply be applied in each layer of the aggregate to yield all of the production terms P_i in eq. 1.

Water transport submodel

Water redistribution in the aggregate was calculated by combining Darcy's law, eq. (2):

$$J_w = -k \cdot \frac{dh}{dx} \quad (2)$$

with the continuity equation, eq. (1), where the production term cancelled because roots were absent. Since only horizontal transport of water occurred in the aggregate, gravitational head was omitted in eq. (2). Both hydraulic conductivity (k) and pressure head of soil water (h) were functions of volumetric water content (θ).

In the experiments (Fig. 4) it appeared that redistribution of water with an initial (schematic) distribution as depicted in Fig. 1b, resulted finally in a non-homogeneous radial distribution of water. This was attributed to the hysteresis phenomenon that may especially occur in the water retention curve (Koorevaar et al., 1983). Preliminary attempts to model the redistribution process of water in the aggregate without taking hysteresis into account failed, because water content was always finally homogeneously distributed. Thus, the submodel for water redistribution had to include hysteresis.

A simulation model describing soil water flow including hysteresis in the water retention curve was reported by Dane and Wierenga (1975). Essentially, the scanning curve for wetting (or drying) was forced to converge to the main wetting (or main drying) curve as a function of the difference between the water content at which a reversal of drying to wetting (or wetting to drying) occurred, and the actual water content. The model was attractive to use because it combined a reasonable description of the hysteresis phenomenon (drawn from tests of the hysteresis function on literature data) with an explicit computational scheme similar to that used in the present simulation model. Dane and Wierenga's model was reformulated so that variable time step integration methods could be used to save computer time. Furthermore, the tabular data input of the main drying and main wetting water retention curves and the hydraulic conductivity–water content curves in the model was replaced by equations described by Van Genuchten (1980). The parameters in these equations were estimated from measured data by procedures outlined by Van Genuchten (1978).

The water submodel is not affected by the results of other submodels, though in real soil hydraulic characteristics may be affected by entrapped air and gas pressure (Chahal, 1966), resulting from gas production and consumption processes; the water submodel, however, directly affects the submodels for the transport processes of solutes and gases.

Solute-transport submodel

Solute transport in the aggregate was calculated by combining eq. (3):

$$J_w = -D_0 \cdot \lambda_w \cdot \theta \cdot \frac{dc_s}{dx} - L_d \cdot |J_w| \cdot \frac{dc_s}{dx} + J_w \cdot c_s \quad (3)$$

with the continuity equation, eq. (1). The three terms in eq. (3) represent Fick's first law for diffusive transport, convective dispersion, and convective transport of the solute, respectively. The diffusive flux in soil is reduced compared with that in free water, because the water phase occupies only a fraction of the soil volume (θ), and the diffusion path has a tortuous geometry (λ_w). The tortuosity factor, λ_w , is an empirical function of the volumetric water content, θ . The convective dispersion coefficient, $L_d \cdot |J_w|$, is linearly related to the average water flow velocity, $|J_w|$ (Bolt, 1979). The concentration of solute (c_s) refers to the water phase of the soil, for no adsorption of solutes to the solid phase is assumed to occur. Numerical dispersion was reduced in the simulation program by computing the convective transport (third term in eq. 3) using the linearly interpolated value for c_s at the transition between adjacent compartments (Goudriaan, 1973).

Equation (3) was derived and extensively discussed by Bolt (1979), and it has been applied in numerous simulation studies, e.g. Leistra (1972, 1980), Frissel and Reiniger (1974), De Wit and van Keulen (1975), Leistra et al. (1980) and Boesten (1986).

It follows directly from eq. (3) that the water flow submodel affects the transport of the solutes as long as the redistribution process continues; diffusive transport, however, will always be present in moist soil, since bacterial activity will hardly ever be similar in adjacent compartments.

Gas-transport submodel

Gas transport in the aggregate was calculated by combining eq. (4):

$$J_g = -D_{ij} \cdot \lambda_g \cdot \epsilon_g \cdot \frac{dc_g}{dx} + J_p \cdot \frac{c_g}{c} \quad (4)$$

with the continuity equation, eq. (1). The first term in eq. (4) represents Fick's first law for diffusive transport incorporating area reduction (ϵ_g) and tortuosity (λ_g), similar to solute transport, but now referring to the gas phase of soil. The binary diffusion coefficients, D_{ij} , were calculated as described previously (Leffelaar, 1987). When eventually pressure changes occurred, the binary diffusion coefficients were pressure corrected. Diffusive transports through the water phase of soil were calculated according to Fick's first law, i.e. the first term in eq. (4), under the assumption that no coupling of gas fluxes will occur in water. This seems plausible, because the main interactions of dissolved gases will be with the water molecules: water density is about three orders of magnitude higher compared with dissolved gas densities.

The second term in eq. (4) is the product of the pressure adjustment flux (J_p) and the relative presence of gas g (c_g/c), that serves to maintain equal total gas pressures on either side of adjacent soil layers. In fact, the second term in eq. (4) embodies the coupling of fluxes in multinary gas mixtures where water is absent (Leffelaar, 1987). The description of diffusion of gases by eq. (4) in multinary, isothermal, isobaric, ideal-gas mixtures, where in principle differences in total gas pressure were caused solely by unequal binary diffusion coefficients (D_{ij}), agreed to within 10% with results of the rigorous gas kinetic theory for such systems (Leffelaar, 1987). The pressure adjustment flux in that case was calculated as the sum of the individual gas fluxes. The integration of the multinary gas diffusion model in the models for water flow and respiration complicates the calculation of the pressure adjustment flux. This is so, because in a wet soil, where water movement occurs, differences in total gas pressure could also be caused by different gas solubilities in water, mass flow of gas due to water movement, and different source/sink terms in adjacent soil layers, aside from the effect of unequal binary diffusion coefficients. To calculate the pressure adjustment flux in the integrated model, the previously used assumption that total gas pressure gradients will not occur in adjacent soil layers (Leffelaar, 1987), was supplemented with the assumption that partitioning of gases over the water and the gas phase is instantaneous and given by the gas solubility coefficients in water. Essentially, the total gas pressure in adjacent gas-continuous layers, i.e. layers that have gas-filled porosities exceeding a certain critical value ϵ_g^{crit} , is kept constant by allowing the gas to flow instantaneously between these layers. ϵ_g^{crit} is assumed to be the value where air permeability is just measurable (Le Van Phuc and Morel-Seytoux, 1972). When the initial distribution of water in soil is such that in the outer layers $\epsilon_g < \epsilon_g^{\text{crit}}$, gas exchange takes place via the water phase, and the total gas concentrations in these layers will undoubtedly be different from the enclosed gas-continuous layers. As water redistributes, layers at the water front will become gas-continuous, and a jump in total gas concentration will appear. When also the outer layer becomes gas-continuous, such a jump in total gas concentration will occur at the interface of the aggregate and the surroundings. The instantaneous adjustment may be compared with the flow of bubbles that sometimes occurs from a swamp or when air from an immersed aggregate is suddenly searching its way out (Stroosnijder and Koorevaar, 1972).

Full details to solve the pressure adjustment flux in the integrated model were given by Leffelaar (1988).

Numerical calculations were done by a program written in Continuous System Modeling Program III (CSMP III) language (IBM, 1975), using the variable time step integration method of Runge-Kutta Simpson, and executed on a VAX machine.

Model parameters

The procedure followed to assess the simulation model was similar to the one proposed by Leffelaar and Wessel (1988): data not measured during the present study were gathered from different authors, and then it was investigated whether it was possible to simulate the overall picture of the experiment by modifying some of the gathered data within reasonable limits.

Aggregate characteristics

The initial volume of the chamber that contained the aggregate for the reported experiment was 530 cm³. Aggregate dimensions were those of the experimental system: height and diameter 2.59 and 9.8 cm, respectively. Fourteen concentric layers were distinguished to characterize the space coordinate of the model aggregate: 5 layers of 0.2 cm, 2 of 0.3 cm, 2 of 0.4 cm, and 5 of 0.5 cm. The choice of both number and grouping of layers was based on a compromise between the need to simulate the expected large gas concentration gradients in the wet outer soil layers with reasonable accuracy, and to optimize the time step used for the Runge-Kutta Simpson integration method both with respect to maintaining numerical stability and to minimize round-off errors in this single precision integration algorithm. Soil porosity, 0.478 cm³ cm⁻³, was calculated from the volume and amount of soil, using a particle density of 2.65 g cm⁻³. Gas pressures in the soil layers and in the chamber that contained the aggregate were initiated according to the gas composition used to flush the respirometer system: 19.525 kPa and 81.8 kPa for oxygen and neon, respectively. Neon was used so that molecular nitrogen from denitrification could be measured. The amount of solution added to the aggregate at the start of the simulation was 43.2 cm³; it contained 3.0 g l⁻¹ of glucose and 8.44 g l⁻¹ of potassium nitrate, so that the amounts of C and N applied were similar to those in the experiment.

Microbiological characteristics

The model also contains a routine to describe anaerobic respiration due to denitrification, the process where nitrite, nitrous oxide and molecular nitrogen are formed from nitrate. The parameterization of the anaerobic part was described in Leffelaar and Wessel (1988), while the parameterization for the aerobic part was given in Leffelaar (1988).

Some essential parameters used are: initial amount of biomass: 10⁻⁵ kg C per kg of dry soil; maximum relative growth rate 0.75 × 10⁻⁵ s⁻¹; the half saturation value for oxygen respiration in water saturated soil crumbs: 10⁻³ mol O₂ m⁻³ H₂O (Greenwood, 1961); half saturation value on glucose: 0.0174 kg C m⁻³ H₂O (Shah and Coulman, 1978); maximum growth yields and maintenance coefficients on glucose as carbon source with oxygen as electron acceptor: 0.503 kg B kg⁻¹ C and 0.212 × 10⁻⁵ kg C kg⁻¹ B s⁻¹, respectively;

similar values for oxygen (needed to calculate the oxygen consumption rate) were $0.65 \text{ kg B kg}^{-1} \text{ O}_2$ and $0.382 \times 10^{-5} \text{ kg}^{-1} \text{ O}_2 \text{ kg}^{-1} \text{ B s}^{-1}$, respectively.

Hydraulic characteristics

Initial water distribution was given by the water content measurement at the start of the experiment, Fig. 4. The main water retention curves and the hydraulic conductivity curves for the sandy loam soil from Lelystad are depicted in Fig. 3. Experimental details were given by Leffelaar (1988). The dashed water retention curves in Fig. 3 were obtained by the optimization procedures by Van Genuchten (1978); the continuous hand-drawn envelope curves produced the best agreement between measured and simulated water contents, however. Therefore, these were used to produce the data presented below. Assuming that the aggregate followed the main drying curve before the solution was added, initiation of pressure head in each compartment was carried out according to the primary wetting scanning curve that is also shown in Fig. 3. To calculate the scanning curves, a hyperbolic type of equation:

$$y = a / (|\theta - \theta_T| + a)$$

was used (Dane and Wierenga, 1975): when the difference between the water content where a reversal from e.g. drying to wetting occurs (θ_T) and the ac-

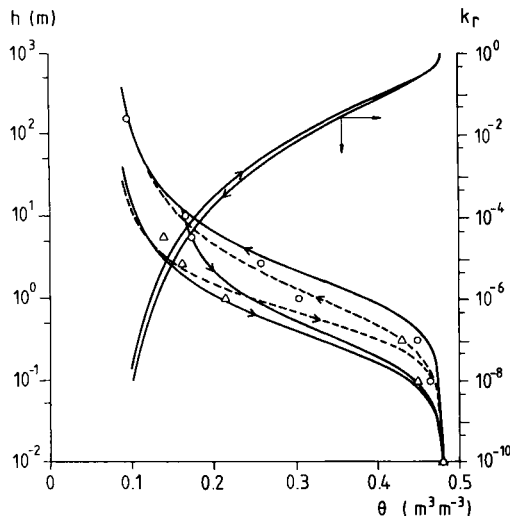


Fig. 3. Experimental ($\circ$: desorption; $\triangle$: sorption), curve-fitted (dashed line, Van Genuchten, 1978), and hand-drawn envelope (solid line) soil water retention curve that produced the best agreement between measured and simulated water contents, and primary wetting scanning curve used for initiation of model; calculated (solid line, Van Genuchten, 1978) relative hydraulic conductivity curves based on hand-drawn water retention curves, for the Lelystad sandy loam.

tual water content (θ) equals a , the scanning curve is halfway between the main water retention curves. The pressure head at the actual water content is then given by the sum of the pressure head at the main drying curve times y , and the main wetting curve times $(1 - y)$. Parameter a in the hyperbola was taken as $0.01 \text{ m}^3 \text{ m}^{-3}$ (Dane and Wierenga, 1975).

The hydraulic conductivity–water content curves in Fig. 3 were calculated from the envelop water retention curves by procedures outlined by Van Genuchten (1978): these calculations gave the relative hydraulic conductivity curves, however, and a matching point was needed, e.g. the saturated hydraulic conductivity. The saturated hydraulic conductivity, $5 \times 10^{-7} \text{ m s}^{-1}$, was obtained following the constant head method described by Kessler and Oosterbaan (1974). Preliminary simulation runs showed that the rate of water redistribution hardly affected the course of other processes since gas-continuity between the aggregate and the surroundings did not occur at the final water distribution. Therefore, solely to reduce computer time, the saturated hydraulic conductivity was taken as 5 times smaller.

Transports characteristics in the water phase

The diffusion coefficient for glucose at 25°C was $6.7 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ (Harremoës, 1978). Diffusion coefficients at about 20°C for oxygen, carbon dioxide, and molecular nitrogen in water were 20×10^{-10} , 16.5×10^{-10} , and $22 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, respectively (Harremoës, 1978). From the preliminary simulation runs it appeared that hardly any gaseous exchange between the interior of the aggregate and the surroundings occurred when these literature data were used. The experimental findings, however, show that exchange of gases between the aggregate and the surroundings was much more pronounced. Assuming that gaseous transports through the water phase of soil were well described, it seems probable that in the experimental aggregate these transports were enhanced, e.g. through small cracks in the outer part of the aggregate or perhaps along the top cover of the soil container (see Fig. 2). In an attempt to take account of this enhancement in gaseous exchange, the diffusion coefficients of gases in water were set 10 times higher compared with the literature values for pure water. Gas solubility coefficients at 20°C and 1 atmosphere for oxygen, carbon dioxide, molecular nitrogen, and neon were reported by Wilhelm et al. (1977). The relationship between the tortuosity factor for diffusion of solutes and gases in the water phase and soil water content were taken from a literature compilation by Leistra (1978). The value for the dispersion length (L_d) for this fine packed soil was suggested by Prof. G.H. Bolt (pers. commun.) as 0.001 m.

Transport characteristics in the gas phase

Parameters and equations needed to calculate binary diffusion coefficients were reported previously (Leffelaar, 1987). As a first approximation, the tortuosity factor for diffusion of gases in the gas phase, λ_g , was represented by

the same relationship as the one used for solutes in the water phase: for the gases it was related to the gas-filled porosity, however. The value for the gas-filled porosity where soil was considered gas-continuous, ϵ_g^{crit} , was taken as $0.063 \text{ m}^3 \text{ m}^{-3}$. This value was simply just sufficient to prevent gas continuity throughout the aggregate. The preliminary simulations showed that when ϵ_g^{crit} was taken slightly smaller, gas continuity occurred and anaerobiosis quickly disappeared: such results were contradictory to the experimental findings and it was thus concluded that no gas-continuity had occurred in the experiment. The value of about 6% for the critical gas-filled porosity was near to experimental values found by Le Van Phuc and Morel-Seytoux (1972), and Corey (1957).

RESULTS AND DISCUSSION

One representative experimental data set on the distribution of water and gases is presented in Figs. 4, 5 and 6 for the Lelystad sandy loam soil, in which the anaerobic process of denitrification, which also produces CO_2 , could occur. Data points originate from the experiments, whereas the lines were produced by the simulation model.

Figure 4 shows the volumetric water distribution at the start of the experiment and at the end after 45 hours. The methods to measure water content were evaluated by comparing the water balance as calculated from the gamma ray attenuation method at the start and the end of the experiment with data from the gravimetric moisture measurement at the end. These yielded vol-

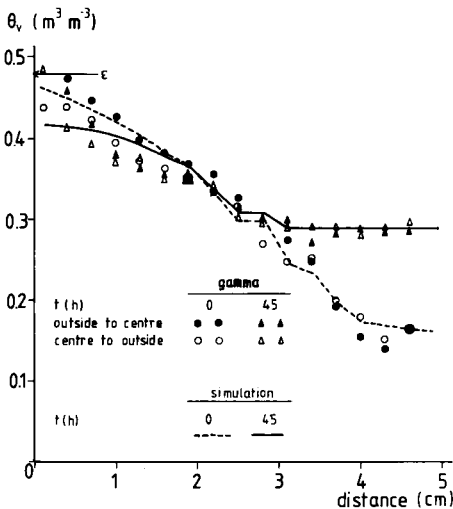


Fig. 4. Experimental and simulated volumetric water distribution in the soil aggregate (center at distance 4.9 cm) at start and end of experiment.

umes of 71.2 ± 0.6 , 72.0 ± 0.6 and 71.0 ± 0.7 ml, respectively, which was considered very satisfactory.

After about 24 hours water redistribution was complete (data not shown), even though a homogeneous volumetric water content (0.363 ± 0.008) was not attained. This indicates that a homogeneous suction distribution was attained in the soil aggregate combined with a heterogeneous water distribution due to hysteresis in the soil water characteristic. In the absence of hysteresis, the air-filled porosity at equilibrium ($0.115 \text{ cm}^3 \text{ cm}^{-3}$) would have been sufficient to let oxygen diffuse into the aggregate and to stop anaerobic processes. In the presence of hysteresis, however, a small amount of added water is sufficient to decrease gaseous diffusion to such an extent, that the oxygen consumption is not met, resulting in anaerobiosis. The volume of air-filled pores in the outer shell of the aggregate remained close to zero, even after 45 hours.

The simulated redistribution of the volumetric water distribution in Fig. 4 was essentially completed after 12 hours. The simulated curve is not smooth, since plotted water contents were obtained by weighing and summing the water contents of the concentric layers that coincided with a measuring location: thus plotted data refer to the rectangular geometry of the water measurements obtained by gamma radiation (De Swart and Groenevelt, 1971). The major portion of the simulated curve and the data points match well. At the outside of the aggregate, however, the simulated water content is about 3% lower than the experimental value, and consequently the air-filled porosity at the outside was that much higher. The critical air-filled porosity in the simulation was so chosen ($0.063 \text{ cm}^3 \text{ cm}^{-3}$), that no gas continuity would occur after the redistribution of water, in accordance with the experimental findings. When the critical air-filled porosity was taken slightly lower, however, the aggregate became gas-continuous in the course of the simulation and anaerobiosis disappeared quickly. It thus appears that air-filled porosity is a determining factor in the regulation of oxygen status of soil, at least at the micro-scale.

Figure 4 shows that the exchange of gases between the soil and the gas collection circuit took place through the water phase, and it was expected that the aggregate would remain anaerobic after the oxygen in its interior was consumed. Figure 5 confirms this expectation. The data points show oxygen pressure in the aggregate center to equal zero after 13 hours, while the peripheral oxygen pressure remains about 2 kPa. Outside the aggregate, however, and after 45 hours, the amount of oxygen had decreased by only 19.5 ml (Fig. 6), corresponding to an oxygen pressure of 15.7 kPa. Oxygen flux can be obtained by differentiating Fig. 6 with respect to time. Oxygen flux density is then obtained by dividing this quantity by the surface area of the aggregate ($79.7 \pm 1.0 \text{ cm}^2$). At 21 hours the oxygen flux density amounted to $2.7 \pm 10.8 \text{ l O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (the large confidence interval is discussed below). This value compares favourably with field measurements in bare soil (Russell, 1973).

Figure 6 also shows the evolution of carbon dioxide. The oxygen and car-

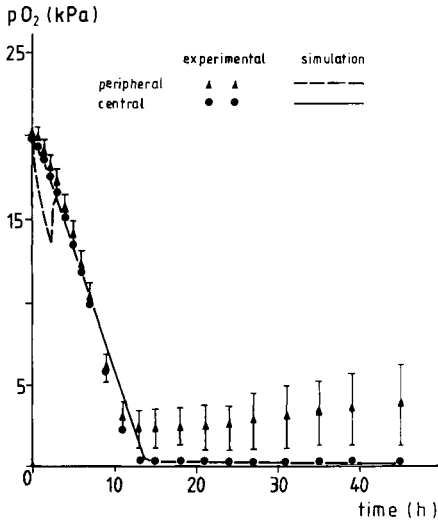


Fig. 5. Experimental and simulated oxygen pressure in center and 4 cm from center (peripheral) of soil aggregate as function of time.

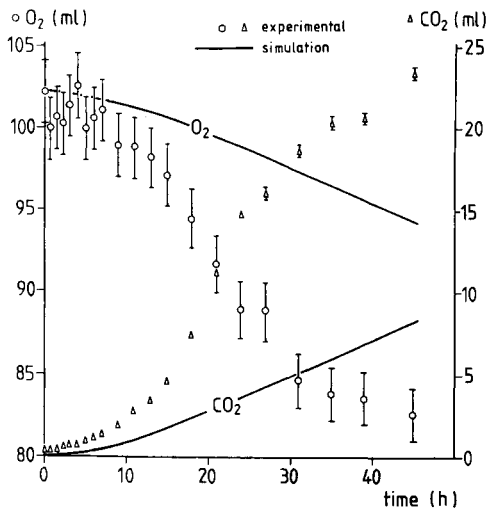


Fig. 6. Experimental and simulated volume of oxygen (left y-axis) and carbon dioxide (right y-axis) in gas collection circuit as function of time.

bon dioxide curves form complementary S-shaped curves. The amounts of oxygen consumed (20 ± 3 ml) and carbon dioxide produced (23 ± 0.5 ml) in 45 hours were practically the same. This resulted in a respiratory quotient, defined as the ratio of the volumetric rates of carbon dioxide production and oxygen consumption, of about one. In a partially anaerobic soil, however,

only a portion of the soil is consuming oxygen, while the whole is producing carbon dioxide and the respiratory quotient is expected to exceed one. Therefore, the respiratory quotient is not a sensitive measure to decide whether a soil is partially anaerobic. Russell (1973) and Gliński and Stępniewski (1985), however, state the opposite with regard to the sensitivity of the respiratory quotient. The CO_2 curve in Fig. 6 was also used to calculate the flux density at 21 hours and yielded $3.7 \pm 1.3 \text{ l CO}_2 \text{ m}^{-2} \text{ day}^{-1}$. This value is of the same order of magnitude as field data of De Jong et al. (1979), though these authors measured CO_2 evolution in a cropped soil. The confidence interval in the O_2 consumption rate is large compared to that in the CO_2 production rate. This is caused by the propagation of the error in the measured amount of O_2 which is determined by the difference of two large numbers. The reasonable error in the measurement of CO_2 makes it a more appropriate measure to assess soil activity.

Figure 5 shows the simulated results of oxygen pressures in the aggregate center and the periphery to equal about zero after 14 h. The oxygen pressure curves show satisfactory similarity to the experimental findings. The experimental results of peripheral (triangles) and central (dots) electrodes coincided up to about 10 hours; after about 14 hours only the experimental results of the central electrode coincided with the simulated results. The simulated results show an interesting feature of the model: when the layer where the peripheral oxygen electrode is located becomes gas-continuous after about 2.5 hours, a pressure jump occurs. Hereafter, the simulated results of both the electrodes coincide. The feature of the pressure jump has a number of aspects. First, the oxygen pressure in the layer that has become gas continuous was about 4 kPa lower than in the adjacent series of gas-continuous layers before the pressure jump occurred; hardly any gradient in oxygen pressure occurred in these adjacent gas-continuous layers. Thus, gaseous transport through the water phase was very limited. Second, this jump or a more gradual change in oxygen pressure was not found in the experimental results. Therefore, it is very probable that internal gas-continuity has been present in the layers where the oxygen electrodes were located in the experiment, resulting in similar electrode readings. Third, such pressure jumps focus attention to the practical problem of measuring oxygen pressure by polarographic Clark-type oxygen electrodes. The calculated absolute pressure in the layer that became gas-continuous was 86.9 and 103.9 kPa before and after the jump, respectively. Since changes in absolute pressure cause proportional changes in oxygen pressure, Clark-type oxygen electrode measurements will be directly affected (Fatt, 1976).

Figure 6 shows that the oxygen outside the simulated aggregate decreased much slower than in the experiment. Oxygen decrease is determined by the respiration rate and the volume of the aerobic outer shell of the aggregate, Fig. 1b. Introduction of higher aerobic respiration rates in an attempt to bet-

ter match the experimental decrease of oxygen in the soil container, however, caused a proportionally faster decrease of the oxygen pressures at the locations of the electrodes. Thus, the enhancement in gaseous exchange between the aggregate and the surroundings was stronger than that attained by the 10 times higher diffusion coefficients in the water phase (see section about model parameters), and a greater part of the soil volume did participate in oxygen respiration in the experiment. The ratio of oxygen flux density at 21 h in the experiment ($2.7 \text{ l O}_2 \text{ m}^{-2} \text{ day}^{-1}$) to that of the simulation ($0.61 \text{ l O}_2 \text{ m}^{-2} \text{ day}^{-1}$) would suggest that the aerobic shell in the experiment was about four times larger than that in the simulation. Since oxygen penetration in the simulation equalled about 0.2 cm, this would mean that in the experiment oxygen penetrated into the aggregate to a depth of about 0.8 cm. This is very near to the peripheral electrode, that in fact stabilized at about 2 kPa in the experiment, Fig. 5.

Figure 6 also shows the evolution of carbon dioxide. It is seen that the simulated curve lags behind compared with the experimental one, similarly as in the case of the oxygen curve. This will be caused in part by the low carbon dioxide transport rate, and in part by a similar reasoning as in the case of oxygen: due to low gas transport rates, the aerobic soil volume participating in aerobic respiration will be smaller, and thus carbon dioxide production under aerobic conditions will be lower, and a smaller amount of this gas is transported into the soil container. Though the simulated curve lags behind compared with the experimental one, it is near complementary to the simulated oxygen curve, resulting in a respiration quotient of about one, similar to the experimental data. In fact, the respiration quotient behaved as a damped oscillation ($0.4 (1 \text{ h}) \rightarrow 1.27 (8 \text{ h}) \rightarrow 1.44 (12 \text{ h}) \rightarrow 0.97 (23 \text{ h}) \rightarrow 1.0 (28 \text{ h})$) and stabilized after 28 hours. One would expect higher final values, however, since the whole of the aggregate was producing carbon dioxide, while only the outer shell consumed oxygen. However, it appeared that a model run in which no anaerobiosis occurred, gave a stable respiration quotient of about 0.45. This implies (1) the aerobic RQ is too low for the organic matter used (glucose) and some of the microbiological parameters should be assessed anew, and (2) an RQ of 1 under partially anaerobic conditions in the model indicates anaerobiosis. When the literature values of the gas diffusion coefficients were used the sequence of respiration quotients was ($0.4 (1 \text{ h}) \rightarrow 0.6 (3 \text{ h}) \rightarrow 0.9 (11 \text{ h}) \rightarrow 1.66 (16 \text{ h}) \rightarrow 2.44 (45 \text{ h})$), and no stabilization occurred. These simulated findings demonstrate a strong influence of transport processes on the respiration quotient of soil. This leads to the conclusion that the respiration quotient is not a sensitive measure to decide whether a soil is partially anaerobic.

No experimental microbiological data could be gathered till now.

CONCLUDING REMARKS

The data presented show that the respirometer system enables us to measure the course in space and time of the state variables which determine the oxygen distribution in soil. For a full explanation of the relationships between the measured data, however, the simulation model describing the interactions between the active biomass, transport processes of water, gases, and nutrients, and storage factors like different solubilities of gases in soil water is needed. Thus, the measurements are gathered to evaluate the simulation model, but the simulation model is needed for a full, or at least a more complete, interpretation of the measured data. The model gives a satisfactory description of the soil biological system studied: part of the experimental results could be described quantitatively, e.g. water distribution and the time course of the oxygen pressure at the experimental positions of the electrodes, whereas other data that deviated from the experimental data, e.g. consumption rate of oxygen and production rates of carbon dioxide, could be understood by studying the dynamic behaviour of the model.

This study gives rise to the following conclusions about a number of physical soil properties that are usually not measured or considered in the soil physics literature with respect to biological processes in soil:

(1) the critical gas-filled porosity, ϵ_g^{crit} , below which gaseous transports take place only through the water phase, is a determining factor in gaseous exchange in soil;

(2) soil water hysteresis is important in causing a non-homogeneous soil water distribution at a homogeneous pressure head distribution, implying that a small amount of added water is sufficient to decrease the gas-filled porosity to values smaller than the critical gas-filled porosity;

(3) water redistribution has a significant effect on the biological processes only in so far as it determines the period during which there are no continuous air-filled pores open to the surface;

(4) soil respiration may cause differences in soil atmospheric pressure in the enclosed gas continuous part of the soil, which may affect soil hydraulic characteristics (Chahal, 1966);

(5) the exchange of gases between aggregate and surroundings is seriously underestimated when it is fully ascribed to diffusion through the water phase of gas-discontinuous soil layers: the model suggests that enhancement of gas exchange occurs, perhaps through small cracks in the outer part of the aggregate or along the top cover of the soil container;

(6) total gas pressure jumps, as simulated by the model, might affect the measurement of oxygen pressure by polarographic oxygen electrodes, though it remains to be investigated whether such pressure jumps will actually occur in field soil.

This study showed that the parameterization of the model is a major prob-

lem that needs attention first. Therefore, a more extensive exploration of both the experimental respirometer system and the theoretical simulation model is intended: the model will be used first to plan experiments with respect to the respirometer system, second to help interpret the data so obtained, and third to investigate the relative importance of a number of parameters in a sensitivity analysis. Furthermore, the model may be used to study trends and interactions between biological activity and transport processes in the aggregate.

In this study the form of the aggregate was mathematically ideal and respiratory activity was homogeneously distributed in the soil. It depended only on environmental conditions that resulted from the interaction of biological activity and transport phenomena. This is why we could apply a model that calculates the dynamics of water, nutrients, oxygen and biological activity as a function of the distance from the outside of the aggregate. In field soils, no mathematically ideal forms exist and the question arises whether soil geometry as we find it in the field should be directly used in modelling, or that for instance an aggregated soil should be idealized in the form of porous spheres, as worked out by Smith (1977, 1980) and Leffelaar (1979), or that it would be most appropriate to develop a soil model that shows no visual, but a physical resemblance with the soil profile. An operational method that enables one to derive a model soil with similar diffusive properties as the soil it originates from, was developed by Rappoldt (1990). The starting point of his method is that for each point in the soil there is a shortest distance to the nearest macropore or crack. The distribution of these distances characterizes the geometry of the soil. A model system can now be derived that has the same distribution of distances as the real soil, but has a much simpler geometry. The model system consists of soil cylinders with different radii: small cylinders represent the soil mass near cracks; the larger cylinders represent the soil mass further away from the cracks. Diffusive transport in the real soil aggregates or structural elements is described then by solving a diffusion equation for the model system. Rappoldt also shows however, that a non-uniform distribution of soil activity seriously disturbs the correspondance between the anaerobic soil fraction and the distribution of distances (Rappoldt, 1992). This means that studies on the process level, as described in the present paper, have to be supplemented with studies describing the distribution of respiratory activity in soil.

APPENDIX

a	half transition constant in hysteresis hyperbola, for explanation see text	$\text{m}^3 \text{m}^{-3}$
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c_i	concentration of substance i . When $i=g$, it refers to gas, and units are either mol m^{-3} of gas phase or $\text{mol m}^{-3} \text{H}_2\text{O}$; when $i=s$, it refers to solute, and unit is $\text{kg m}^{-3} \text{H}_2\text{O}$	amount m^{-3}
c	total molar gas concentration in general, $c = \sum_g c_g$	mol m^{-3}
C_i	concentration of substance i with respect to volume of soil. When $i=g$, it refers to gas, and amount is mol; when $i=s$, it refers to solute, and amount is kg; when $i=w$, it refers to water, and amount is $\text{m}^3 \text{H}_2\text{O}$. Symbol is only used in the equation of continuity, eq. (1).	amount m^{-3} of soil
D_0	diffusion coefficient of solute or gas in free liquid water	$\text{m}^2 \text{s}^{-1}$
D_{ij}	binary diffusion coefficient of gas pair $i-j$ in free air	$\text{m}^2 \text{s}^{-1}$
h	pressure head of soil water. h is a function of volumetric water content	m
J_i	flux of substance i . When $i=g$, it refers to gas, and amount is mol; when $i=s$, it refers to solute, and amount is kg; when $i=w$, it refers to water, and amount is $\text{m}^3 \text{H}_2\text{O}$	$\text{amount m}^{-2} \text{s}^{-1}$
J_p	pressure adjustment flux	$\text{mol m}^{-2} \text{s}^{-1}$
k	hydraulic conductivity. k is a function of volumetric water content	m s^{-1}
L_d	dispersion length parameter	m
P_i	net production term of substance i . When $i=g$, it refers to gas, and amount is mol; when $i=s$, it refers to solute, and amount is kg	$\text{amount m}^{-3} \text{s}^{-1}$
p	pressure	Pa
R	gas constant	$\text{Pa m}^3 \text{mol}^{-1} \text{K}^{-1}$
T	absolute temperature	K
t	time	s
x	space coordinate	m
y	weight factor: outcome of hyperbolic equation that forces a scanning curve towards one of the main water retention curves ($0 \leq y \leq 1$)	—
ϵ_g	volumetric gas content	$\text{m}^3 \text{m}^{-3}$
ϵ_g^{crit}	critical gas content where gas phase in layers is just discontinuous	$\text{m}^3 \text{m}^{-3}$
θ	volumetric water content	$\text{m}^3 \text{m}^{-3}$
λ_i	tortuosity factor. When $i=w$, tortuosity in water phase; when $i=g$, tortuosity in gas phase. λ_w and λ_g are functions of θ or ϵ_g , respectively	—
	bars around a variable means absolute value	—

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Round Table on water movement, oxygen supply, nutrient supply, and biological processes on the micro-scale

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In this Round Table three themes were discussed.

Theme 1

When explanatory models on aeration in homogeneous soil are developed, the dynamics of soil micro-organisms is usually included. Therefore physiological parameters, such as relative growth rates and maintenance coefficients, are needed. Unfortunately, these are difficult to separate from the influence of the soil physical and chemical characteristics on micro-organisms. This raises the question whether it is meaningful to measure physiological parameters and soil physical and chemical parameters separately or whether all parameters can be measured in the soil system.

Anderson, Germany: Separate studies of physical and physiological parameters are not very useful. If you want to understand processes at the microbial community level or if you want to compare microbial communities in different soils, you need the habitat of the soil for physiological parameters.

Ingham, USA: She agrees with Anderson. In laboratory cultures availability of C, N, and other nutrients tends to be high and the resulting growth rates are meaningless relative to soil conditions. Soil scientists should give detailed descriptions of the habitats for various organisms in the soil. The biologists can then tell which transformations can occur, possibly causing changes of the habitats. Soil scientists and biologists have to interact.

Chairman: You seem to agree that physiological parameters have to be studied in situ.

Smiles, Australia: Biologists have to provide information to the physicists. Fluxes and concentrations cannot be described, unless the boundary conditions at interfaces of actively respiring organisms and the soil system are specified. This is a challenge for the biologists.

Van Breemen, The Netherlands: The clustering of cells on agar plates and in soils is very different. So ideally physiological parameters should be determined in the soil habitat. But it is doubtful that this can be done. Physicists and biologists keep throwing the ball to each other, but it should stick long enough to be worthwhile.

Chairman: Are you thinking of adhesion of organisms?

Anderson, Germany: Physiological parameters should be determined at the microbial community level. Comparison of maintenance coefficients *in vitro* and *in situ* shows that there is luxury consumption *in vitro*.

Chairman: Rijnaarts and his colleagues of the Department of Microbiology of Wageningen Agricultural University have compared the functioning of free organisms and organisms adhered to teflon spheres, using parameters in the Monod equation as the basis for comparison. The specific growth rate of the adhered organisms first followed the Monod equation with a small half saturation value, but later slowed down. The free organisms followed the Monod equation initially, with an order of magnitude larger saturation value, and were poisoned at higher concentrations.

Theme 2

Field soils may contain large biopores and cracks. Preferential flow in macropores may contribute to heterogeneity of the distribution of water and nutrients. Decomposable organic matter is usually also heterogeneously distributed. Soil physical properties generally have widely different values at different scales and as a result the inherent characteristic lengths and times of processes are also scale dependent. This raises the question, how should heterogeneity be dealt with in connection with soil aeration and water movement.

Bouma, The Netherlands: One should not lump (1) macropores and cracks in structured soils, (2) distribution of the decomposable organic matter content, and (3) distribution of water and nutrients resulting from preferential flow paths. Rather these aspects should be treated in a sequential manner. Roots often tend to follow cracks and other large continuous pores and this, apart from the influence of soil tillage, determines strongly the distribution of decomposable organic matter. Macropores and cracks in structured soils may lead to preferential flow. Such flow has its characteristic dynamics and techniques are available now to monitor it. In his lecture Altemueller pointed out that we should watch out with regarding aggregates as discrete entities. The size distribution resulting from sieving depends on the procedure followed, longer sieving gives more smaller aggregates. If you look at an undisturbed soil in a thin section you often (not always) see a matrix with larger pores in it. It may then be more meaningful to look at characteristic patterns near larger pores.

Chairman: It is not our intention to lump separate causes but to indicate various aspects of the causes of heterogeneity.

Box, USA: In the nineteen sixties the soil physicist Grable used maize seed germination to demonstrate at various soil bulk densities that soil aeration becomes a problem at the air entry value (A.R. Grable and E.G. Siemer, 1968. Effect of bulk density, aggregate size and soil water suction on oxygen diffusion, redox potentials and elongation of corn roots. Soil Sci. Soc. Am. Proc., 32: 180–186). Later Flühler considered some probabilistic aspects of this problem and showed that for germination aeration becomes a problem in the range of suctions of 70 to 100 mb (H. Flühler, L.H. Stolzy and M.S. Ardakani, 1976. A statistical approach to define soil aeration in respect to denitrification. Soil Sci. 122: 115–123). This would not be very different for microbes. Therefore an evaluation of the air content of aggregates under various circumstances may provide a simple and direct approach to the aeration problem.

Crawford, UK: Avoid making distinction between macro and micro structures. Rather concentrate on trying to understand the scaling relationships, that is on the functions relating phenomena at different scales. This is the approach used in the poster on gas movement in structured soil.

Rappoldt, The Netherlands: If you have an experimental aggregate with a simple geometry and if the biologists can give all the necessary parameters you can try to describe the situation in full detail (see paper by Leffelaar). Such studies may clarify the nature of the processes. If you have soil heterogeneity at different scales and if you have heterogeneous aggregates you have to give up on a completely deterministic approach, if not for fundamental reasons then definitely for practical reasons, considering the data needed to describe the structure. Homogeneous aggregates may have anoxic centers, heterogeneous aggregates may have scattered anoxic regions, depending on the location of the decomposable organic matter. What we need is a clever choice of macroscopic parameters and establishment of empirical relationships among such parameters.

Chairman: An example of such an approach is the description of biodecomposition of a soil pollutant as a first order decay process, requiring just a decay constant. Of course, for microbiologists such an approach may not be very exciting.

Raats, The Netherlands: Detailed studies at smaller scales may be important. Crawford rightly suggests that one always should keep the adjoining scales in mind. Sometimes the study of phenomena at a certain scale yields information about phenomena at smaller scales. A prime example is the information about pore size distribution that can be obtained from studying retention and movement of water at the Darcy–Richards scale. Often one studies processes at a small scale and next wishes to integrate to a larger scale. The work of Rappoldt is a good example. It has been remarked that large scale phenomena are rather dull. However, large scale phenomena become more interesting if they have built-in information from smaller scales.

Along a growing root you get quite a succession of organisms. Root growth has been successfully modelled by considering a coordinate system attached to the tip of the growing root. If the root is growing at a constant rate, then in terms of such a coordinate system the process is steady. Silk, among others, has done a lot of work describing root growth in this fashion and she also has analyzed a variety of biochemical processes in roots (see e.g. W.K. Silk, 1984. Quantitative descriptions of development. *Ann. Rev. Plant Physiol.*, 35: 479–518). It appears that this approach could also be used to formulate a population dynamics model for succession organisms along roots. Has that been done?

Dighton, UK reacts and says he has not actually developed such a sequential model. But he has studied ecto-mycorrhiza colonization of roots. He looked at interaction between species and colonization patterns in space and time, considering relative growth rates of both roots and the mycorrhizal fungi. To understand the processes in the ecto-mycorrhizal sphere, the bacterial populations have to be considered also. The difficulty is again to determine the necessary physiological parameters at the small scale of interest. The impasse is that the biologists do not yet have the necessary technologies.

Van Noordwijk, The Netherlands: Darrah (1991) (*Plant and Soil*, 133: 187–191, 1991 and 138:147–158) studied the microbial population dynamics along a growing root producing exudates. It was found that if the root grows slow enough, the microbes might respond before the root arrives, whereas if the root grows fast, the microbes might respond after the root tip passes.

Crawford, UK: The group of Lynch at Queens College (London) is also looking at microbial dynamics near an exuding root tip. Their theory suggests that the dynamics of the population are chaotically unstable.

So far there appears to be not yet any experimental backup for that prediction.

Theme 3

Soil animals have a certain power to change soil structure and with that soil characteristics, e.g., parameters governing transports of water and gases, and the soil penetrability for roots. The time scale at which these processes take place is long (years). Traffic and soil tillage will partially destroy these structures. How can management techniques, such as soil tillage, be implemented so that existing positive effects from soil animals are conserved or promoted? What is the time period that animal-made holes and structures will exist?

McKenzie, Australia Some answer to the second question is contained in the poster on earthworms. At 10 cm depth they last only a few days, whereas at 20 cm depth they may last for up to 2 years.

Marinissen, The Netherlands: The questions posed are difficult and very wide. The answers depend on which species one is talking about. One has to know what animals do. Only when that is known can one look at persistence of holes and possibilities for managing the situation.

Chairman: Can the action of animals render soil tillage superfluous? How do crop yields on tilled and not tilled soils compare?

Koehler, Germany: The question only considers the direct effects of the animals. Soil structure is also promoted by indirect effects of soil animals. It is difficult to distinguish between animal made holes and structures which are influenced by activity of animals at a wide range of spatial and temporal scales, down to the scale of microbes.

Chairman: The question arises from two physicists and that may explain why it is rather wide.

Anonymous: Regarding the first question, one should ask not only biologists, but also farmers. Without tillage, a lot of money may have to be spend on herbicides. Under dry conditions, drought may be more severe without tillage. In general yields are the same without tillage.

Rappoldt, The Netherlands: The presence of various species may imply a range of characteristic times for the persistence of biopores. However, one can also focus on overall response times. If one has tilled a long time and stops, it is of most interest to know how long it takes to reach a new steady situation.

Box, USA: In studies over a period of 15 to 17 years comparing tillage and non-tillage or direct-drill in the southeast of the USA, yields of soybeans and grain sorghum after a few years of non-tillage were just as high as with conventual tillage. In this region, similar results are expected for cotton and maize.

Marinissen, The Netherlands: Results will depend on the climate and the crop rotation. In the rather wet climate of the Netherlands and with a narrow rotation including sugar beets and potatoes, soil tillage cannot be missed.

Translation of soil features across levels of spatial resolution — Introduction to round table discussion

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INTRODUCTION

This final round table is comprised of several parts: first, a general introduction covering several points of interest in the general topic: Translation of features in the soil across landscapes. Specific points raised include: techniques for studying distributions of soil biota, statistical distributions, fractals, and ecology and evolution. This is followed by a general discussion, and then specific statements by the chairman, in the role of “agent provocateur”. Discussion follows each of the points raised.

DISTRIBUTIONS OF SOIL BIOTA

We are trying to go across many levels of spatial resolution. At a finer scale, one can follow bacterial movement through soils. We want to know a great deal about what happens in the individual microsites, and movements into micropores and macropores. Research on very up-to-date analytical procedures that have been pursued in the soil microfabric are interesting; e.g., there are techniques using enzyme specific stains (Foster, 1985) which didn't even get covered in our poster session. For a good review of soil biota and effects on soil structure, see Jastrow and Miller (1991).

This section merely summarizes some of the kind of things such as distribution and abundance in terms of faunal impacts on soil microfabric. I would hope that perhaps there will be some way we can tie what goes on in the earthworm macropore channels with the hydrologic implications.

When we consider the non-destructive types of analysis, it would be most helpful for soil biologists to have some way to actually scale-up from the microsites. For future studies it would be useful to get some idea of what are abundances of organisms and some of their products, and what happens with

their lateral distribution, i.e., effective transfers within soil layers. The very effective Oregon State University (VCR) Tape talked about a wide range of macro- and meso-arthropods which can have a significant impact on transport of propagules.

Regarding non-destructive studies, mycorrhiza researchers are making use of not only CAT-scanning but other appropriate remote sensors for determining Ca content in situ in the soil (K. Cromack Jr. et al., pers. commun., 1991).

STATISTICAL ANALYSES

Means, variance, skewness and kurtosis. What are the consequences of faunal and root growth and further action, on the major soil structural features of interest. Two of the posters noted that there may be a reduced variance due to faunal influences on certain aspects of soil processes. If there are many more soil macropores a large amount of water will flush through them, and not in the adjacent smaller pores.

You can measure random, regular and aggregated distributions, of soil organisms or their structures (i.e., burrows or macropores). How often do you observe these? If there is a significant territoriality a regular distribution is often assumed. Quite often soil organisms are highly aggregated. Does this indicate something important to us? I think it actually does if we can put it together with some other important measures of central tendency, particularly the variance as well as the mean.

FRACTALS

I'm going to discuss a little more about fractals because it seems to me that if we are doing scaling we have to think about something in addition to the regular aspects of what is called scaling theory.

Fractals are merely something that gives you some idea of self similarity of events or distributions at a particular scale (Fig. 1). Several of these ideas and figures come out of an interesting Soil Science Society of America book on scaling problems in soil physics (Hillel and Elrick, 1990) and also Tyler and Wheatcraft (1990a), and Wheatcraft and Tyler (1988). You can begin at a particular low level of resolution working your way up and at a non-fractal basis, seeing increasingly more and more (Fig. 1). With fractals there is a tendency to see more and more and more at a particular level. We must be careful of the dimensions of reference objects, e.g., a camera lens cap (Fig. 2). My point is that we need absolute reference points to be able to compare scaling features at given levels and as we move between scales.

One of the concerns of people studying water flow in soils is that at a certain scale (meters) there is increasing tortuosity — there is more and more channeling. The tortuosity is not linear, but increases at some incredible rate, ex-

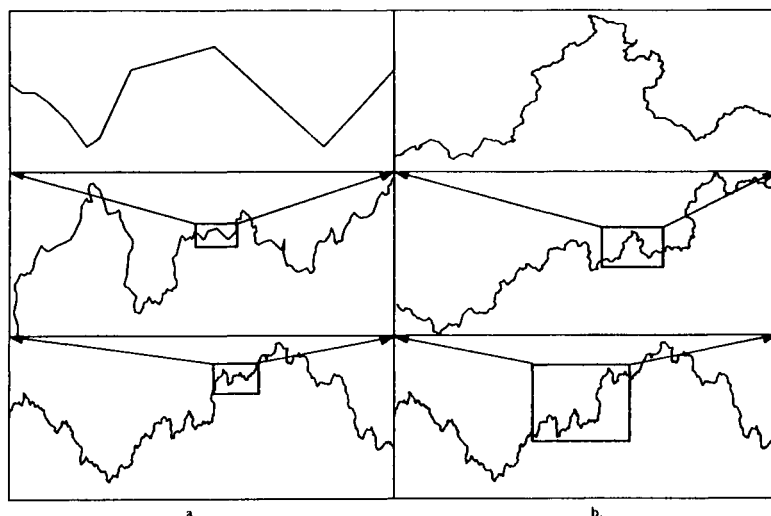


Fig. 1. Representations of (a) nonfractal scaling, and (b) fractal scaling, showing increasingly complex microrelief in (b). (From Tyler and Wheatcraft, 1990b.)

pressible in fractals. For dispersivity of water there can be an actual scaling factor of greater than one hundred (Fig. 3). I am trying to indicate that by keeping our eyes open, and doing the appropriate field sampling, we could make some important advances. The conceptual model (Fig. 4) is a brief summary which shows a magnified soil section situated along some toposequence, with biotic and hydrologic events embedded in the landscape matrix.

ECOLOGY AND EVOLUTION IN SOIL SYSTEMS

I am an ecologist. So I love to coin words. In this conference, we've had syn-chronicity, syn-localisation, and I will add: syn-evolution or coeno-evolution. D.S. Wilson in 1980 asked: "Do soil fauna and their associated microflora", he was particularly asking about earthworms, "have a virtual co-evolution of their various functions?" This can indeed be considered community evolution and most, of the more purist evolutionists will say: "No, this is absolutely unacceptable. There is not any type of kinship selection."

If we think of the soil as an external rumen in which there are many interesting interactions going on, can we say that we have involved a "cast" of thousands? Peter Price (1991) has talked about the sort of thing we should be thinking about in terms of syn-biotic events. Price added up radiating macro-organisms, associated micro-organisms and species number (Table 1). Now there's all sorts of talk about the fact that cyanobacteria and fungi and some land plants got together and produced a very interesting landflora. Plants and fungi have of course a reason. I think we can say "of course". Many peo-

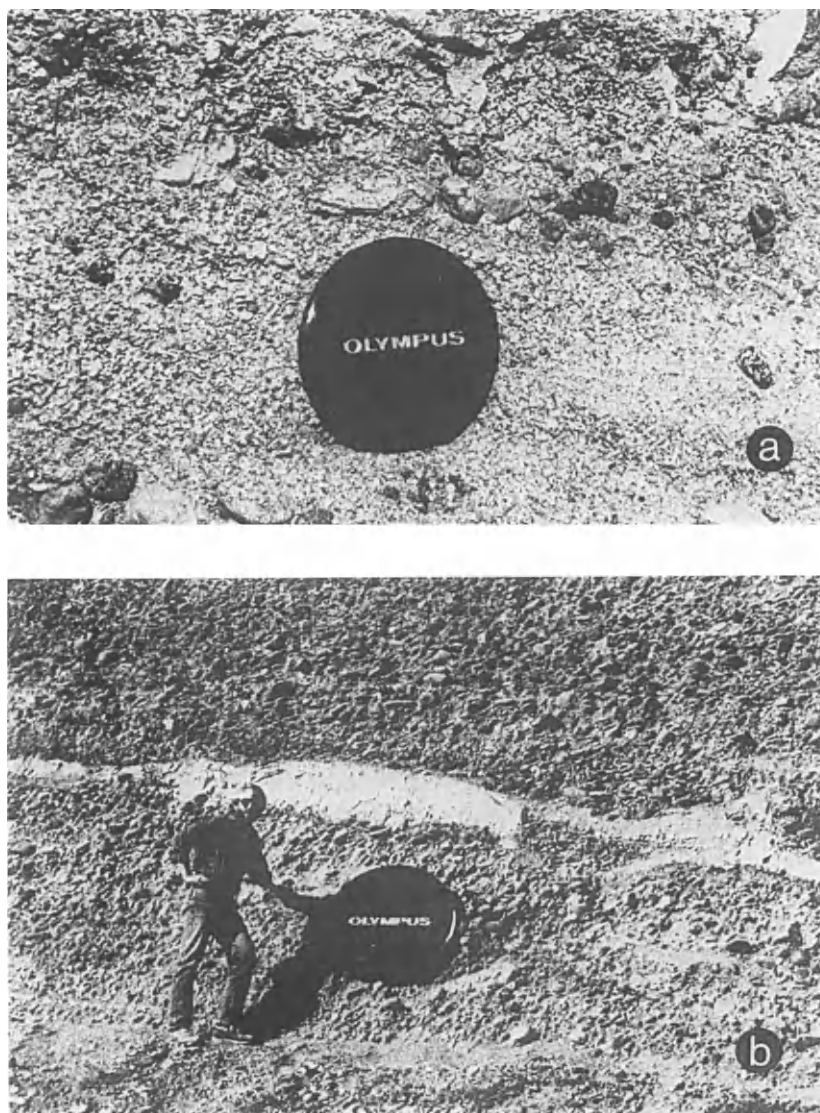


Fig. 2. (a) Close-up of soil profile; (b) geologic outcrop, as larger view of the same profile. (From Tyler and Wheatcraft, 1990b.)

ple now accept: this is a very important symbiotic event with a lot of co-evolution (Pirozynski and Malloch, 1975). The same is true for insects and micro-organisms associated with various invertebrate herbivores.

What is the situation in the soil for our knowledge of mutualism? Damn little. We've got earthworms, microarthropods, myriapods, other invertebrates. They are undoubtedly setting up or making use of some quite impor-

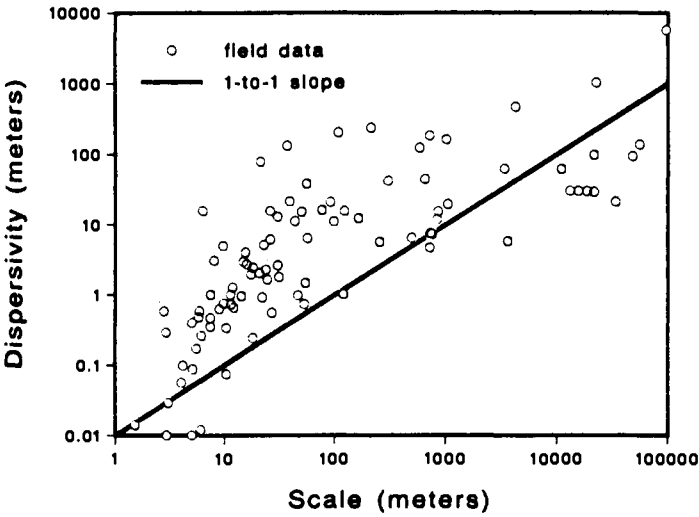


Fig. 3. Dispersivity versus scale of experiment, illustrating 10 to 100:1 ratios of dispersivity to scale in meters. (From Wheatcraft and Tyler 1988, after Gelhar et al., 1985.)

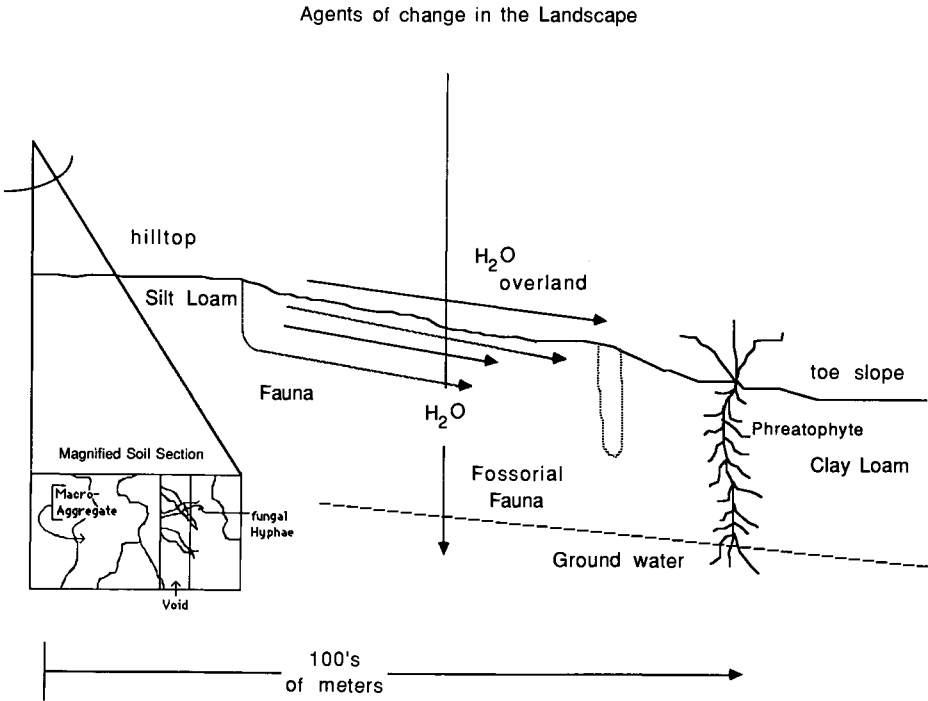


Fig. 4. Agents of change in the landscape, ranging from the level of macroaggregates (1–2 mm) to hundreds of meters. (From Coleman et al., 1993).

TABLE 1

Major adaptive radiations, evolving into mutualisms (modified from Price, 1991)

Radiating macroorganisms	Associated microorganisms	No. of Species	Percent of species on earth
Land plants	Aquatic algae and fungi	300,000	21
Land plants	Plants and fungi as mycorrhiza	280,000	20
Invertebrate herbivores	Insects and microorganisms	360,000	26
Parasitic wasps	Endoparasitic wasps and viruses	100,000	7
Vertebrate herbivores	Artiodactyla and bacteria	200	$\ll 1$
Earthworms, microarthropods, myriapods, other invertebrates (nematodes, etc.)	Bacteria, microfungi, viruses?	??	??

tant symbiotic associations. How are we going to find them if we do not go, indeed, beyond the biomass (J. Dighton, pers. commun., 1991)? And how do we do that? Jim Lynch pointed out in this meeting, let's use some appropriate molecular tools to identify some of the microbial colonies in microsites. I'm willing to bet you people that we're going to find mutualistic associations. In fact Joke Marinissen and her colleagues have been doing some interesting work on what kinds of bacteria and fungi are associated with earthworm burrows. Is there a chance that there is co-evolution going on in soils?

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OPEN FLOOR TO ROUND TABLE DISCUSSION

Raats, The Netherlands: I was very pleased to see all your comments on the fractals and the illustrations taken from soil physics, from Scott Tyler and Stephen Wheatcraft. I think what I've heard about fractals, that's a very good thing. A lot of these examples we've seen have been fractal geometries. And I just would like to mention that I think it is also important to study processes on fractal geometries and we see the beginnings of that, for example soil physicists have been deriving water retention curves on fractal pore spaces. In the last issue of the Soil Science Society of America Journal there are again two interesting papers on that. Usually, on the retention curve there come out power functions for the relationship between the water content and the pressure head. That is very pleasing because that's a model that has been used since the mid-fifties. Until a few years ago it was a purely empirical model. There is similar work on the hydraulic conductivity of a particular fractal geometry. That is when you take spheres and you fill the holes between the spheres again with spheres and go on like that. On the other hand, I think, it is also important to study the genesis of the fractals and again in the sphere where I work for example people look at cracking patterns and the origin of that and why perhaps they should be fractal. Another important area at the moment is models for unstable infiltration. Petroleum engineers have worked there earlier, but again in water movement in unsaturated soil and in leaching patterns often you get very irregular penetration and in some cases people find that you get fractal patterns. And models out of the sphere of fractals like diffusing limited aggregation can be used to explain those.

Lussenhop, USA: I would like to react to the last table you put on the overhead from Peter Price, showing that the soil biologists haven't done their share of demonstrating close associations between micro-organisms and invertebrates by arguing that this maybe is especially difficult to do. I think that it is easy enough for terrestrial specialists to see these associations to demonstrate them, because of the adaptations for transport for example on the part of the invertebrates, whereas in the soil, if you are an invertebrate you are living in a sort of sea of micro-organisms. In fact, you might be the reciprocal problem of being eaten by micro-organisms if you are not careful. So, I think that demonstrations that such close associations exists are going to be more difficult as a result.

Barois, Mexico: On the last point I also would like to say that we did some work on earthworms and relationships with microflora and we've measured along the intestinal gut and the cast microbial activity. We see that there is a very high relationship between microflora and Oligochaeta and we make an analogy with the rhizosphere and drilosphere. We think that sometimes the earthworm when it secretes intestinal mucus or cutaneous mucus it is the same as exudates from the roots.

Van Noordwijk, The Netherlands: I would like to return to the soil biologists in the audience. We need something equivalent to the scaling aspects of soil physics. I suggested home range as an equivalent for animal ecology. We've been talking about micro-, meso- and whatever fauna we have on the basis of their body size and diameter, but in terms of the dynamics of the system a more interesting classification would be on the size of the home range. And of course in soil we have fauna acting in response to the heterogeneity. There are organisms which are just sitting there and switch on or off depending on whenever something happens, and there are organisms who are running around and are able to go to the place where the things are happening. I do think the dynamics of that sort of interaction would bring us quite a bit further. And the question is, we're dealing with interactions between organisms

which have quite a different home range so to speak. And that's a typical thing for a structured soil which leads to heterogeneity. There is some similarity with predator-prey interactions on that level which come out with real different dynamical situations than assuming that everything is perfectly mixed.

Tomlin, Canada: I was wondering how many levels of scale or orders of magnitude of scale we have to cross in order to prove the theory or to disprove application of fractals. The reason I ask the question is that ecology has been plagued over the years with the problems of having different descriptive techniques come and go, and will fractals end up in the same case of being inadequate descriptors of situations that obtain in ecology?

Crawford, UK: It is important to specify the length scales. I am not extrapolating beyond these length scales of which fractal properties have been established.

Secondly, I also think that fractal geometry has its limitations, particularly the fractal dimension on its own does not by any means give a full characteristic of the soil and geometry.

Raats, The Netherlands: Yes, I would like to add to this. One example I mentioned earlier is that if you take a model of spheres and the holes you fill in with spheres, that model can be used to calculate hydraulic conductivity. Of course, if you would take a real fractal you would fill...well, it would be solid and the conductivity would be zero. You have to cut it off somewhere.

Coleman: Here is a general statement, as a null hypothesis, I am throwing out for comments from the audience.

Question/Statement 1

Apart from measurements of infiltration, and water flow, there are no other significant measures of translating soil features across levels of resolution in soil systems.

McKenzie, Australia: I would have thought that some of the strength measurements have also crossed the scales. We've used a range of penetrometers from certainly millimeter-scales up to penetrometer measurements on centimeters and pushed all sorts of probes and things in the soils. And so, I think, soil strength is one thing that has crossed some of the boundaries.

Elliott, USA: I would suggest even just measurements of soil organic matter might actually translate across levels of scales, especially if you relate them to some sort of hierarchical aggregate structure where measures at one level can relate either to levels higher than that or measures at one level given knowledge of a particular system can be translated to levels lower than that.

Raats, The Netherlands: You didn't mean it seriously. I think, at least when you look at other transport processes the situation is not really different. You mean, that one gets so much attention. But work is being done on solute transport where this happens also. One example is: the different models that people used for root activity on different scales.

Dighton, UK: If you take your analogy a bit further, all you're interested in is scales of resolution. Do we really have to worry about the organisms? I was always looking at processes and you may have a process that is occurring within a bacterium which is doing one job and you may have fungi doing a very similar process at larger scale of resolution and you have the whole soil doing the same process at another resolution. Can we just ignore biotic components and just go back to black box theories and just stack one black box on top of another?

Crawford, Dundee: No, is the answer. Because the idea would ignore the interactions occurring on the smaller scale, which are very important for the global behaviour of the system.

Coleman: Does anyone else want to have any comments possibly on global behaviour? Maybe we can go on to Question No. 2.

Question/Statement 2

Plant physiologists, oceanographers and meteorologists have explained global change trends. Do soil scientists/biologists have any significant contributions?

Ingham, USA: Yes, we have some very important contributions to give to global climate change, for example the plant physiologists, meteorologists suggest that southern pine in USA which now grow in the southeast will migrate and will now grow farther North, perhaps in Michigan. So, in another 30 or 40 years our southern pine plantations will be in Michigan instead of Alabama. The problem with that is whether currently southern pine seedlings or seed cones will make it to Michigan. They will germinate and begin to grow in Michigan well, but they will never make it beyond June or July because they're killed by pathogens in that soil. The effect is the soil is much more buffered than the above-ground portion of the system. So, it is not likely that the pathogens in the Michigan soil are going to disappear with global climate change, with the change of one or two, or even four or five degrees of air temperature change. So, southern pine may try to move up to Michigan but in fact they won't survive, which means that Michigan will now be grassland. So, from a biological point of view, we have a lot to offer.

Tomlin, Canada: Another one that comes to mind immediately is the hypothesis that methane production from the reduced ocean level shoals when most of the sea water is tied up in the glaciers in fact induces by global warming the next melting round. Obviously, if that was to be true that will be a significant contribution on the part of soil scientists.

Rusek, Czechoslovakia: Not only soil scientists but also soil biologists can contribute to the IGBP programme. Not all of us have long term studies on soil biota, but there are some studies. We have data from IGBP, and from the Man and the Biosphere Programme. I'm working in a SCOPE programme Ecotones and there is a chapter in the book about global changes reflected in alpine and subalpine ecotones and ecosystems, which is based on long term studies in the Tatra National Park in Central Europe. The changes which occurred there in the last few years are on such a scale that we can call them an ecological catastrophe. I was surprised at how such marked changes could develop during certain years. I was studying different soil parameters, plant communities with a group of biologists from 1958 until now. Up to 1977 nothing changed in the soil and in the plant communities, etc. But now, from 1977 to 1991 during this time such deep changes in soil biota communities occurred, dominant species changed completely. Some dominant species are no more living there in these areas affected by long distance transfer of acid rain. But it is not only acid rain, it is also a change in the amount of precipitation, snow cover duration, etc., etc. Some soil horizons in alpine belt disappeared, as a result of leached fulvic acids. And it is only in the last ten years.

Brussaard, The Netherlands: Nico van Breemen has just left, but if he were still here he would probably argue that most of the greenhouse gases evolve from the soil. Do soil scientists and thus soil biologists have any contributions? Clearly, yes.

Another point is that we can make an interesting comparison with the research on acidification as compared to the research on global change. In the acidification research programmes, at least here in the Netherlands, the research was dominated by plant physiologists, meteorologists, environmental chemists and soil chemists. And they seemingly thought that they could explain what was happening. But only in the end it became clear that a lot of the transformations were of a biological nature. And apart from some work on nitrification and denitrification under acid conditions there has been very little soil biology research involved. A lot of the transformations, as we all know, are not only of a microbiological nature but the soil fauna do contribute a lot there. And it may well be that the effects of global change in terms of more carbon dioxide and other greenhouse gases may cause changes in

the decomposition process that in the first place we can't predict because as yet we don't understand the effect on the individual species. And if we don't understand them the next question is the species that could deal with the decomposition process under the changed conditions; will they be there? Because they have to expand their ranges or they are differentially affected in any case.

Rusek, Czechoslovakia: In the frame of the IGBP-programme there is established now or is establishing a net of terrestrial observatories. And soil biologists should ask to attend for monitoring the soil conditions, the soil biota conditions, the soil processes conditions in almost each country which is participating in IGBP. They are establishing such long term monitoring stations, terrestrial observatories. We have to tell the people, meteorologists, pedologists, it is very important to know what is really occurring in sites and how it will change in the future. Soil biologist-microbiologists, zoologists etc. should be in all such ecological monitoring systems.

Brian, Canada: I think the soil scientist has a lot to contribute, but before we can contribute fully, I think we have to venture away from our institutions a bit and travel to sites with large storages of carbon. I'm suggesting tundra environments, very peaty environments. The agricultural soils are not the important key here. We're looking at muskegs, taiga and tundra, where those huge storages are. We have to get out there and to study these dynamics and not the traditional soils which we have been used to studying.

Coleman, USA:

Question/Statement 3

Considering movement of pollutants and impacts, fish and earthworms are the only indicators of any importance.

Wright, UK: Since you bring up fish, you are talking about marine aquatic systems. There is a system called BNP which take some vertebrate species in different rivers and basically order them in a scoring system. Certain species are indicative of pollution, and certain species are indicative of cleaner waters. And that has been successfully used as a scoring system. You get scores at the end of it and basically it says whether your river is clean for biological life or not, depending on what species are there. It is not just earthworms or fish.

Brussaard, The Netherlands: I think there is good biological reason why this statement can't be true. If you look at the foodweb models, we've seen several during this workshop, you see that the soil organisms are aggregated in so-called functional groups. And that is done among others based on their physiology. Environmental pollutants and contaminants often have very specific physiological effects. So, various groups of soil organisms are affected differently based on their physiology and the type of contaminant you talk about. So, if these groups are differentially affected, this may affect the whole functioning of the system, depending on of course how important those various groups are for the functioning of the system. In some cases they may be relatively unimportant depending on the processes you talk about. In other cases they exert key functions. So, it really depends on the type of contaminant, on the physiology of the functional group and relative importance of the functional group for the functioning of the system as a whole.

Laanbroek, The Netherlands: Well, I guess that this statement does not only provide an opportunity for everybody to plead for his own taxonomic group as being extremely important indicators, but I think that the problematic point is the last words: the 'indicators of importance'. Quite a few people probably tend to think of important indicators as organisms that suggest that something in the environment is becoming worse for us humans. But there are quite a lot of ecosystem functions for the organisms themselves if you consider all the kinds of mutualisms which might be as important as the survival of number of humans, at least in the most polluted sites in Northern Europe and America.

Reddy, India: In addition to the fishes there are quite a number of aquatic invertebrates in the sediments of various fresh water ecosystems, which are good indicators of pollution. Thus dipteran larvae are indicators of acute organic pollutants. Thus, there are many different lentic macroinvertebrate groups which are recognizable as bioindicators of pollution.

Tomlin, Canada: The best indicator of pollution is probably the presence of humans.

Juma, Canada: I want to go back to microbial biomass. We have indications, if you think of acid rain pollution, that as soon as the environment of the microbial biomass under natural conditions is changed to acidic conditions, we find that the release of carbon per unit biomass increases. So, this unit of microbial biomass can be used as an indicator of stress.

Dighton, UK: I wonder, are we slow in our response time and are we talking about pollution here and the impact of soil organisms on pollution or as possible pollution indicators? I remember when the whole world was going mad on acid rain and sulphur dioxide pollution and more recently on ozone pollution the soil ecologists really seem to be slow off the mark. When we started to act together and said we want to do experiments and we started to get all these fancy facilities for doing photosynthesis and respiration and everything else, we said: "Hey we want to look at SO₂ as a pollutant", they threw their hands up and said: "Oh no, we've done that. We are now on greenhouse gases." And the poor soil scientists haven't caught up. When Chernobyl blew we had the problem there of radiocesium contamination. Everybody went screaming around because we had "hot" sheep. We were more concerned with plant-animal transfers and nobody considered the soil as an ecosystem and what was happening there. It is only now that we are beginning to understand perhaps the importance of some of the organisms within the soil that are maybe contributing to long term locking up or mobilization in the soil. Now, Chernobyl is no longer in vogue, and it is global climate warming. Is our response time too long?

Uvarov, Russia: Maybe I should come into both previous questions. I think that only recently we've come to the conclusion that the functioning of land ecosystems is a function of the relationships between soil biota, soil micro-organisms, soil fauna, roots and soil structure. I believe that it is highly important to pay our attention to the study of this functioning. And maybe then we shall be able to understand more easily the functioning of polluted or disturbed ecosystems.

Coleman, USA: Thank you everyone for your attention. Our session is adjourned.